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Profiles of plasma estrogens, progesterone and their metabolites after oral or vaginal administration of estradiol or progesterone

Khalil Nahoul, Louis Dehennin, Michel Jondet and Marc Roger

Fondation de Recherche en Hormonologie, 67–77 Boulevard Pasteur, 94260 Fresnes (France)

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Doses of 100 mg of micronized progesterone (P) and of 0.5 mg of micronized estradiol (E_2) were administered vaginally and orally, respectively, in the early follicular phase of the menstrual cycle in six premenopausal women. In the second cycle, the same doses were administered in the same subjects, orally for P and vaginally for E_2. Serial blood samples were collected and the following steroids were assayed by highly reliable techniques: P, E_2, estrone (E_1), deoxycorticosterone (DOC), 5α- and 5β-pregnanolone and the sulfates of E_1, E_2, and DOC. Circulating P and E_2 levels were higher after vaginal than after oral administration, while those of E_1 were similar after either route. Metabolites of P (DOC, DOCS and pregnanolone) were higher after oral administration. Concerning estrogen sulfates, E_1S concentrations were similar whichever the route, while those of E_2S were lower after oral than after vaginal administration. This study has confirmed that metabolism of ingested P and E_2 occurs mainly in the intestine. Moreover, P was predominantly metabolized to 5α-reduced derivatives, whatever the route of administration. In view of the metabolic pathways which are operative and of the peripheral plasma levels which were found, the vaginal route appears to be more adequate than the oral one for hormone replacement therapy.

Key words: pharmacokinetics; progesterone; estradiol; estrone; deoxycorticosterone; pregnenolone; sulfates

Introduction

Since replacement therapy with estradiol (E_2) and progesterone (P) is aimed to restore a physiological status, the plasma concentrations attained should be within the normal range. As the plasma levels depend on the route of administration (oral, vaginal, transdermal, sublingual and intranasal), several pharmacokinetic studies have been devoted to these types of E_2 [1–3] and P [3] administrations, which were performed in a rather small number of subjects. In addition, the techniques used for the determination of P were not specific enough to guarantee reliable results in the presence of large amounts of metabolites [4]. Moreover, high intra- and inter-

Correspondence to: K. Nahoul, Fondation de Recherche en Hormonologie, 67–77 Boulevard Pasteur, 94260 Fresnes Cedex, France.

individual variabilities of the circulating levels were found after either route of administration and thus the prediction of the plasma concentration after a given dosage was rather difficult. This makes also that the comparison of the different routes of administration might be uneasy.

The aim of this work was to establish and compare plasma steroid patterns, using reliable techniques, after administration of P and E_2 to the same subjects by oral and vaginal routes. Moreover, this study was not restricted to unconjugated estrogens and P but included the determination of estrone (E_1), 11-deoxycorticosterone (DOC), 3α-hydroxy-5α-pregnan-20-one and its 5β-isomer (5α- and 5β-pregnanolone) as well as the sulfates of E_1, E_2 and DOC. Thus it is expected that the efficiency of the oral and vaginal routes might be more accurately compared since the two types of treatment were performed in the same subjects.

Materials and Methods

Subjects

Six normally cycling women (age: 25–35 years) without oral contraceptive medication, volunteered for this study which was performed between days 1 and 5 of two successive cycles. The early follicular phase was chosen because the ovarian production of estrogens and P is minimal and minor changes of the circulating levels can more easily be evidenced.

Protocol

Whichever the treatment or the route of administration, the study was always performed in fasting subjects and breakfast was taken 2 h after medication.

In the early follicular phase (days 1–5) of the first cycle, 100 mg micronized progesterone (Utrogestan®, Laboratoires Besins Iscovesco, Paris) were inserted into the vagina between 0800 and 0900 h (time 0P). The second day a tablet of 0.5 mg of micronized E_2 (Laboratoire Theramex, Paris) was administered orally (time $0E_2$).

In the second cycle, the same dose of P was administered orally in the morning (time 0P), in the early follicular phase and on the following day, the same dose of E_2 was inserted intravaginally (time $0E_2$).

Blood samples were collected on EDTA before and 2, 4, 6 and 24 h after each administration. Plasma was separated after centrifugation and kept frozen at −20°C until assays were performed.

Methods

Radioimmunnoassays. Unconjugated steroids were determined after chromatographic separation of plasma extracts performed either on Celite columns for P and DOC [5] or on Sephadex LH-20 columns for E_1 and E_2 [6]. In the latter case, the residue obtained after evaporation of the diethyl ether extract was dissolved in methylene chloride/methanol (95:5, v/v) and applied on Sephadex LH-20 column (8.5 × 170 mm) prepared in the same solvent mixture. Elution was also performed with this mixture. The first fraction (11 ml) was discarded, the second (4 ml) eluted E_1, the third (5 ml) was discarded and the fourth (7 ml) eluted E_2. The two eluates were evaporated to dryness under a stream of nitrogen and the residues were analyz-

ed by RIA with the same antiserum and the corresponding tracer as described previously for P [5].

Steroid sulfates were determined by the same methods as above after removing the unconjugated steroids by preliminary solvent extraction and subsequent solvolysis according to Aso et al. [7].

The techniques were thorougly validated and the intra- and interassay variabilities did not exceed 10% for P and the estrogens but were 13.3% and 7.6%, respectively for DOC at a mean level of 47 pg/ml [6].

Gas chromatography-mass spectrometry. The determination of 5α- and 5β-pregnanolones was performed by gas chromatography-mass spectrometry with stable isotope dilution [8]. Briefly, known amounts of deuterium labelled analogues were added to plasma samples (0.3 ml or less) which were then equilibrated and extracted. The extracts were purified by liquid chromatography on Sephadex LH-20, derivatized and selected ion monitoring was performed at nominal masses *m/z* 496 and 500, corresponding to the characteristic ions of the heptaflurobutyrates of the native and the labelled pregnanolones, respectively. Intra-assay variability was between 3 and 5% for concentrations of 1 ng/ml or more and between 7 and 10% for concentrations below 0.5 ng/ml.

Statistical analysis. Results were expressed as the arithmetic mean ± S.E.M. (standard error of the mean). After testing the normality of the sample distributions with Kolmogorov-Smirnov and Wilk-Shapiro tests, statistical differences were evaluated using parametric or non-parametric paired tests [9].

Results

Progesterone administration

Plasma progesterone (Fig. 1). The vaginal administration was followed by a rapid rise of P levels: a peak was observed in four subjects at 0P + 6 h and in the two others at 0P + 24 h. However, the mean maximum P concentration (4.7 ± 0.8 ng/ml) (mean ± S.E.M.) occurred at 0P + 6 h and the mean level observed at 0P + 24 h (4.5 ± 1.1 ng/ml) was not significantly different from the peak value. The levels decreased gradually afterwards, yet the value reached at 0P + 48 h was still higher than baseline level but the difference was not statistically significant (Table I).

After oral administration, mean P levels peaked at 0P + 2 h (1.5 ± 0.2 ng/ml), then decreased rapidly so that the mean concentration observed at 0P + 6 h was not significantly different from the baseline value (Table II). In comparison with the levels observed after vaginal administration, those obtained after the oral route were much lower (the mean peak value was about three times lower and occurred earlier).

Plasma pregnanolone (Fig. 2). Baseline levels of the 5α-isomer were higher than those of the 5β-isomer in all the subjects. The pattern displayed by these two metabolites after vaginal P administration indicates only a slight and delayed increase of the 5α-isomer. No change whatsoever could be observed for the levels of the 5β-isomer, which was generally undetectable (Table III).

After oral P administration, there was a marked increase of both isomers with a peak at 0P + 2 h (14.00 ± 2.30 ng/ml and 3.55 ± 0.30 ng/ml for 5α- and 5β-pregnanolone, respectively; $P < 0.01$). The levels of 5α-isomer were markedly higher than those of the other isomer (Table III).

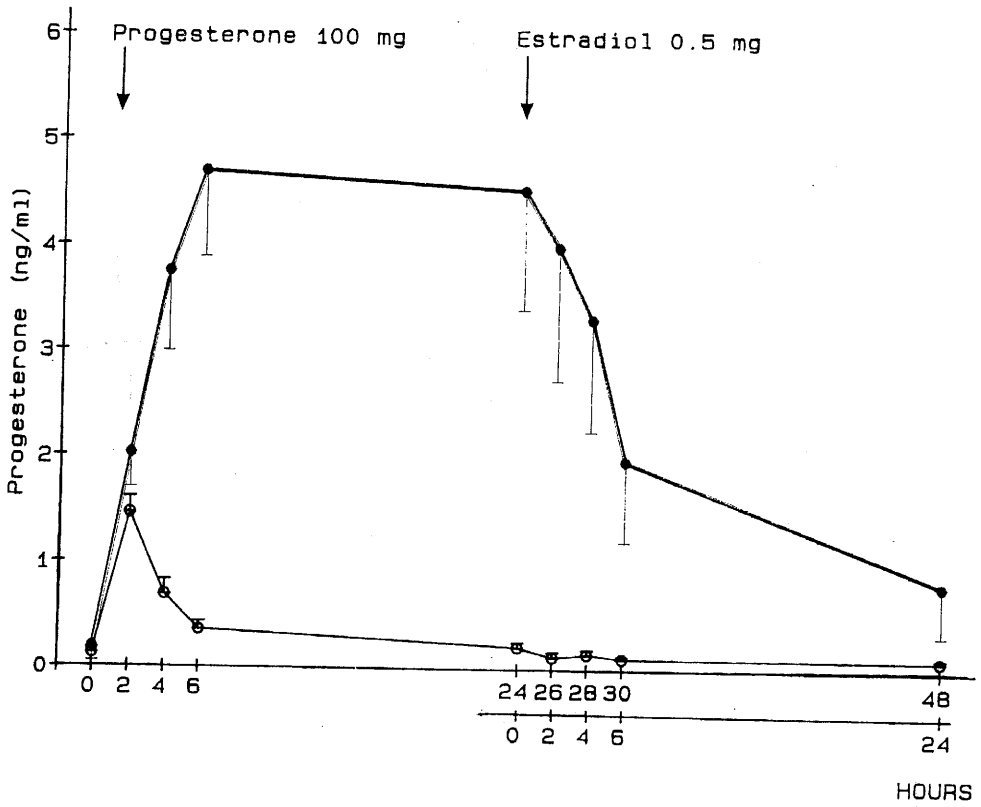

Fig. 1. Plasma levels (mean ± S.E.M.) of progesterone after vaginal (●) and oral (○) administration of progesterone.

Plasma deoxycorticosterone (Fig. 3). DOC levels increased inconstantly after vaginal administration. In fact, rapid DOC elevation was observed in only three subjects, while for the others no significant variation could be evidenced in two of them, the third subject had a significant increase at 0P + 24 h. However, the mean peak level (98 ± 51 pg/ml) occurred at 0P + 4 h (Table I) but it was not significantly different from mean baseline value. At 0P + 24 h the levels were similar to baseline values, then they decreased gradually until 0P + 30 h and increased again at 0P + 48 h. These variations are related to the circadian rhythm of DOC.

When P was orally administered, there was an increase of DOC levels in all subjects with a peak occurring at 0P + 2 h and reaching a value which was on an average about sixfold higher than baseline levels (676 ± 189 pg/ml) ($P < 0.01$) (Table II). This peak was followed by a rapid decrease with a mean level at 0P + 24 h similar to baseline value. Thereafter, the profile of DOC levels corresponded to the circadian rhythm of the steroid, similarly to what was observed after vaginal administration.

The comparison of DOC levels between the two protocols shows that the oral route resulted in higher values.

Plasma deoxycorticosterone sulfate (Fig. 4). A slight increase of DOCS levels was

TABLE I

PLASMA UNCONJUGATED STEROID LEVELS (MEAN AND (RANGE)) AFTER VAGINAL ADMINISTRATION OF PROGESTERONE ($t = 0$) AND ORAL ADMINISTRATION OF ESTRADIOL ($t = 24$ h).

Time (h)	P (ng/ml)	DOC (pg/ml)	E1 (pg/ml)	E2 (pg/ml)
0P	0.19	33	50	120
(P vaginal route)	(0.05–0.90)	(10–55)	(22–67)	(34–228)
2	2.00*	78	75	162
	(0.80–2.95)	(29–169)	(54–101)	(21–306)
4	3.80*	98	66	118
	(0.90–5.80)	(16–350)	(38–105)	(40–185)
6	4.70*	67	61	125
	(0.90–5.70)	(30–189)	(44–73)	(29–220)
24 (0E2)	4.50**	45	57	138
(E2 oral route)	(0.05–7.60)	(10–123)	(22–116)	(21–209)
26	4.00†	42	191†	186‡
	(0.05–7.90)	(10–108)	(83–419)	(99–294)
28	3.30†	40	241**	211‡
	(0.05–6.75)	(10–93)	(130–456)	(96–332)
30	2.00‡	28	225**	155
	(0.05–5.30)	(10–49)	(114–409)	(86–260)
48	0.80	48	118	123
	(0.05–3.10)	(10–99)	(15–219)	(53–196)

*$P < 0.001$. **$P < 0.01$.
†$P < 0.02$. ‡$P < 0.05$.

also noted in all subjects when P was administered vaginally yet the mean value of the small peak observed at 0P + 2 h was not significantly different from baseline level. A second peak was evidenced in all subjects the next day at 0P + 28 h or at 0P + 30 h. However, though the mean levels observed at these times were higher than baseline levels, the differences were not statistically significant ($P = 0.07$).

Oral P administration resulted in an increase of DOCS in all subjects reaching values three- to tenfold the baseline levels The mean peak value (267 ± 40 pg/ml; $P < 0.01$) was observed at 0P + 2 h. In addition, a second lower peak occurred, in all subjects, at 0P + 28 h and its mean value (175 ± 34 pg/ml) was significantly higher than mean baseline levels ($P < 0.05$).

In any case, the levels were lower after vaginal than after oral administration.

Estradiol administration

Plasma estradiol and estrone (Figs. 5 and 6). The mean basal E_2 and E_1 plasma levels were relatively high. In fact, only one of the six subjects had elevated estrogen concentrations and no obvious cause was found for this finding.

The oral administration of 0.5 mg of micronized E_2 resulted in an increase of E_2 levels which peaked at a mean value of 211 ± 35 pg/ml at $0E_2$ + 4 h (Fig. 5). Similarly there was an increase of E_1 levels with a peak occurring also at $0E_2$ + 4 h

TABLE II

PLASMA UNCONJUGATED STEROID LEVELS (MEAN AND (RANGE)) AFTER ORAL ADMINISTRATION OF PROGESTERONE ($t = 0$) AND VAGINAL ADMINISTRATION OF ESTRADIOL ($t = 24$ h).

Time (h)	P (ng/ml)	DOC (pg/ml)	E1 (pg/ml)	E2 (pg/ml)
0P	0.13	116	52	83
(P oral route)	(0.05–0.35)	(45–356)	(22–73)	(35–158)
2	1.50*	676**	58	84
	(1.00–2.10)	(145–1435)	(25–80)	(22–140)
4	0.70*	192†	49	80
	(0.25–1.25)	(62–392)	(22–83)	(24–128)
6	0.36**	112	57	77
	(0.05–0.60)	(35–207)	(29–90)	(36–165)
24 (0E2)	0.21	124	38	90
(E2 vaginal route)	(0.05–0.40)	(24–258)	(10–81)	(27–202)
26	0.12	123	161**	2714*
	(0.05–0.30)	(28–321)	(88–243)	(1812–3492)
28	0.15	106	269**	2687*
	(0.05–0.30)	(41–347)	(115–441)	(1650–4145)
30	0.11	67	237**	1110**
	(0.05–0.20)	(24–164)	(128–443)	(583–2055)
48	0.11	106	98	121
	(0.05–0.20)	(10–312)	(33–230)	(18–300)

*$P < 0.001$. **$P < 0.01$
†$P < 0.05$.

and reaching a mean value of 241 ± 51 pg/ml (Fig. 6). Thus the E_1 peak was slightly higher than that of E_2 (Table I).

In comparison with baseline levels, the increase of E_1 was higher than for E_2. This becomes more obvious when the ratio of E_1 to E_2 levels was calculated. Indeed, there was a significant increase of this ratio to reach a maximum at $0E_2 + 6$ h (1.80 ± 0.65). Thereafter the value of the ratio decreased and at $0E_2 + 24$ h it was similar to that observed before oral administration of E_2 (Fig. 7).

The E_2 levels attained after vaginal administration were markedly more elevated than those observed after oral ingestion and the peak was reached earlier at $0E_2 + 2$ h (Table 2, Fig. 5). In fact, the mean peak value (2713 ± 313 pg/ml) was about twelvefold higher than that of the peak found after oral administration. There was a gradual decrease afterwards, but the mean level observed at $0E_2 + 24$ h was still higher than the mean baseline level, yet the difference was not significant.

Concerning E_1, the mean peak level (269 ± 46 pg/ml) was comparable to that observed after oral administration and the patterns were similar whatever the route of E_2 administration (Fig. 6).

The marked increase of E_2 levels resulted in a E_1/E_2 pattern different from that observed after oral administration. Indeed, the profile of this ratio after vaginal administration was the mirror image of that observed after E_2 administration by the other route, showing a nadir at $0E_2 + 2$ h (0.06 ± 0.01) (Fig. 7).

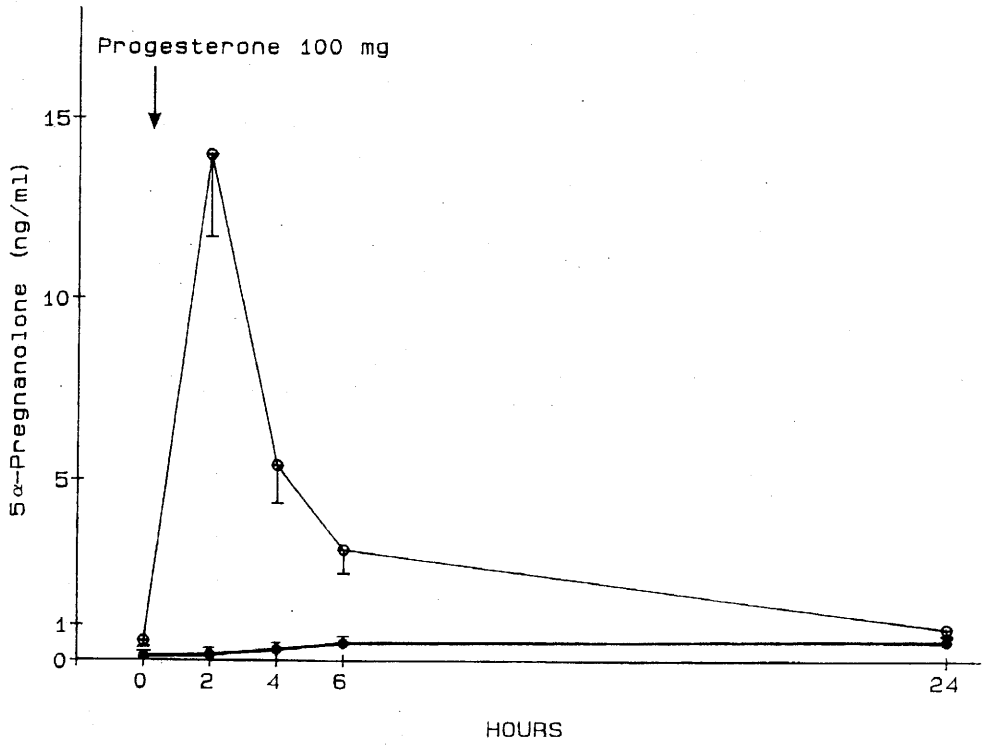

Fig. 2. Plasma levels (mean ± S.E.M.) of 5-pregnanolone after vaginal (●) and oral (○) administration of progesterone.

TABLE III

PLASMA 5α- AND 5β-PREGNANOLONES (5α-P-ONE AND 5β-P-ONE) LEVELS (MEAN AND (RANGE)) AFTER ORAL OR VAGINAL ADMINISTRATION OF PROGESTERONE.

Oral administration			Vaginal administration	
Time (h)	5α-P-one	5β-P-one	5α-P-one	5β-p-one
	Oral	Vaginal	Oral	Vaginal
0	0.55 (0.18–1.05)	0.12 (0.05–0.23)	0.11 (0.05–0.15)	ND†
2	14.00* (7.46–22.80)	0.16 (0.05–0.25)	3.55* (2.71–4.91)	ND
4	5.38* (2.30–8.08)	0.31** (0.10–0.48)	1.20* (0.94–1.86)	ND
6	3.07* (1.77–4.62)	0.48* (0.05–0.74)	0.76** (0.27–1.04)	ND
24	0.89** (0.51–1.50)	0.55* (0.05–0.87)	0.17 (0.05–0.40)	ND

*$P < 0.01$. **$P < 0.05$

†ND: not detectable (<0.05 ng/ml).

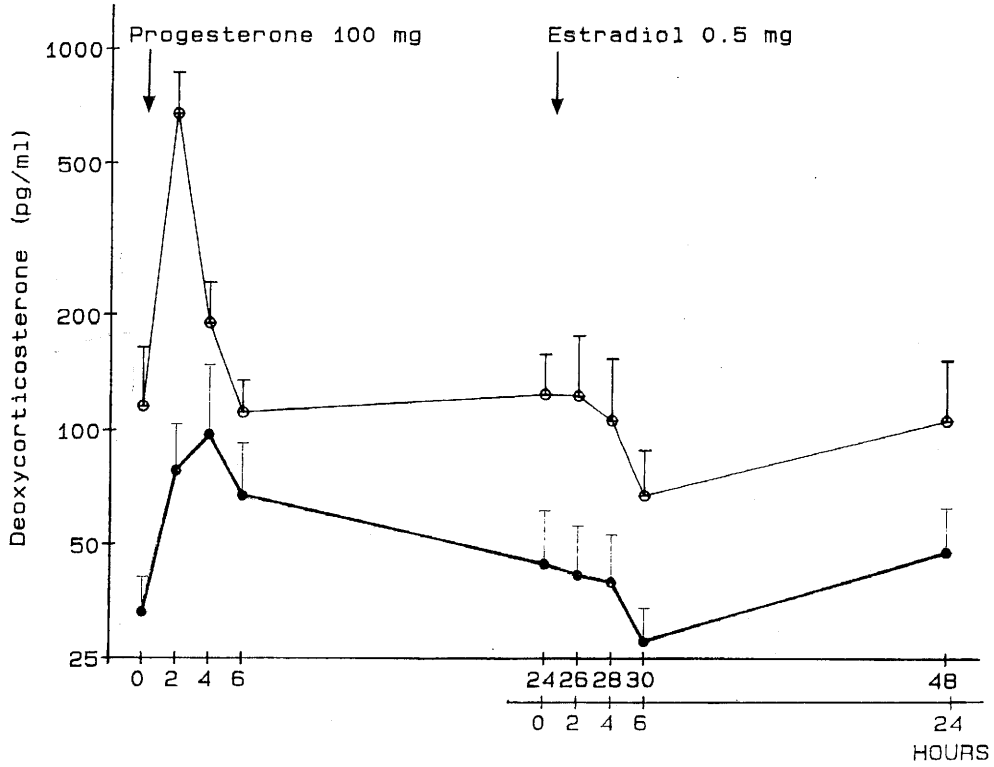

Fig. 3. Plasma levels (mean ± S.E.M.) of deoxycorticosterone after vaginal (●) and oral (O) administration of progesterone.

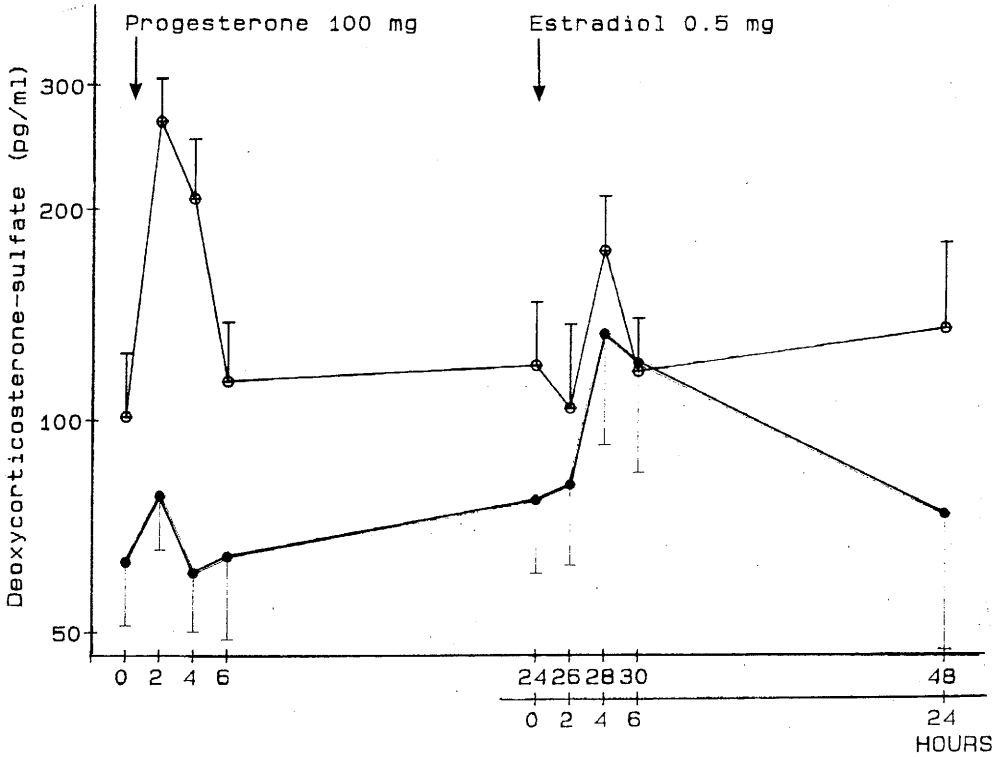

Fig. 4. Plasma levels (mean ± S.E.M.) of deoxycorticosterone sulfate after vaginal (●) and oral (O) administration of progesterone.

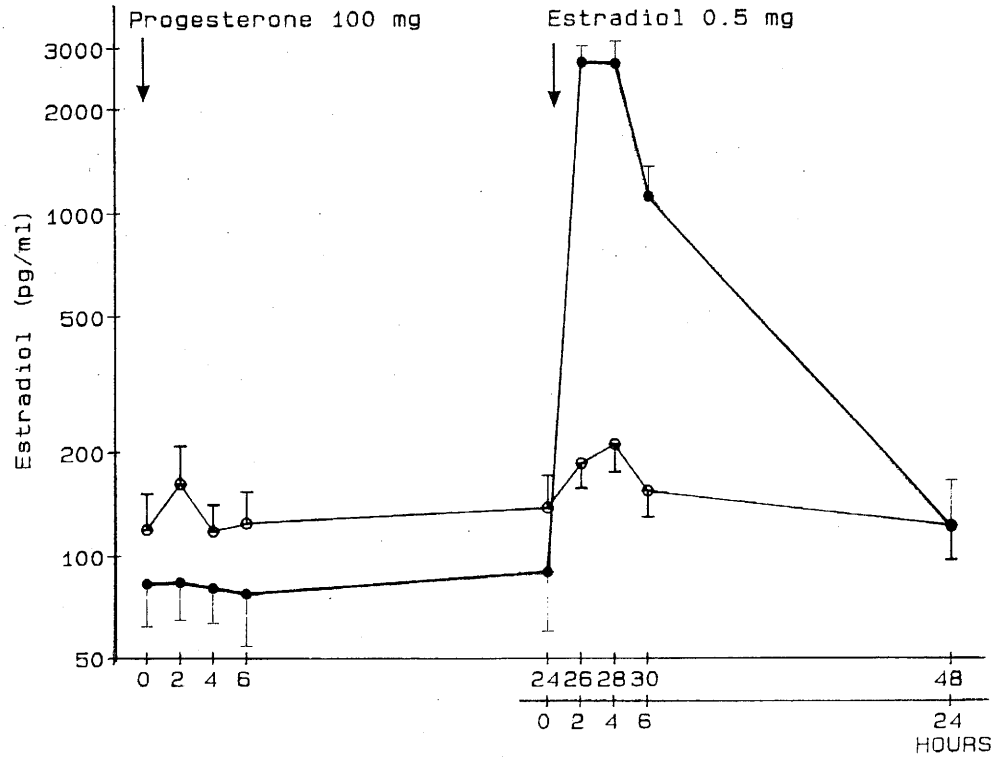

Fig. 5. Plasma levels (mean ± S.E.M.) of estradiol after vaginal (●) and oral (○) administration of estradiol.

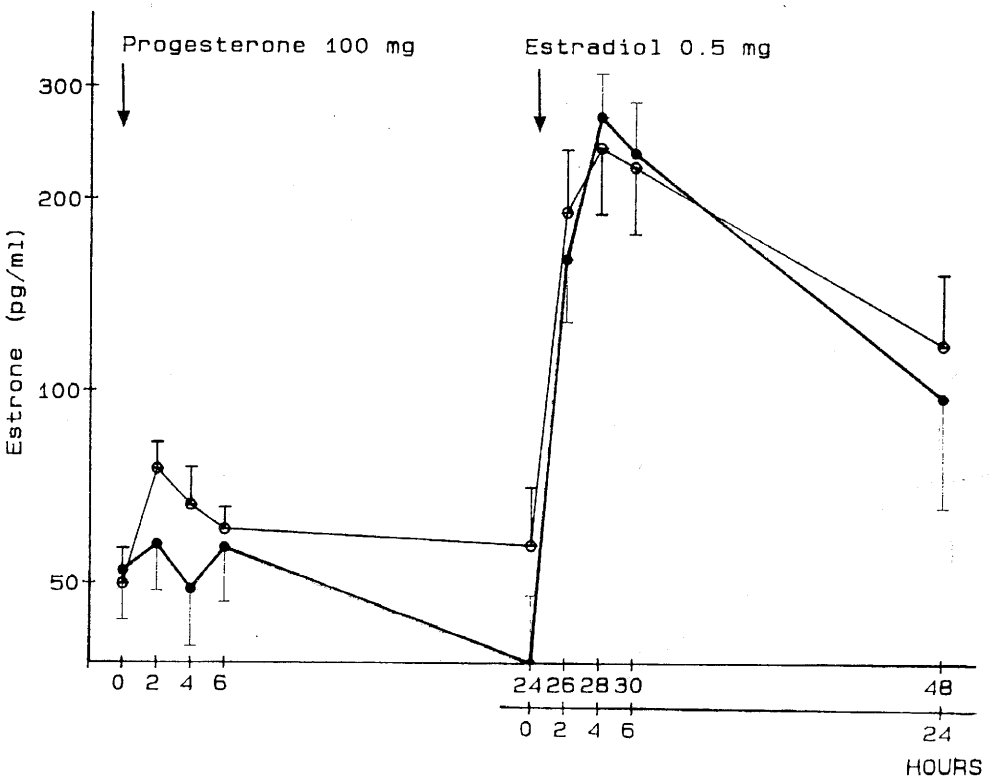

Fig. 6. Plasma levels (mean ± S.E.M.) of estrone after vaginal (●) and oral (○) administration of estradiol.

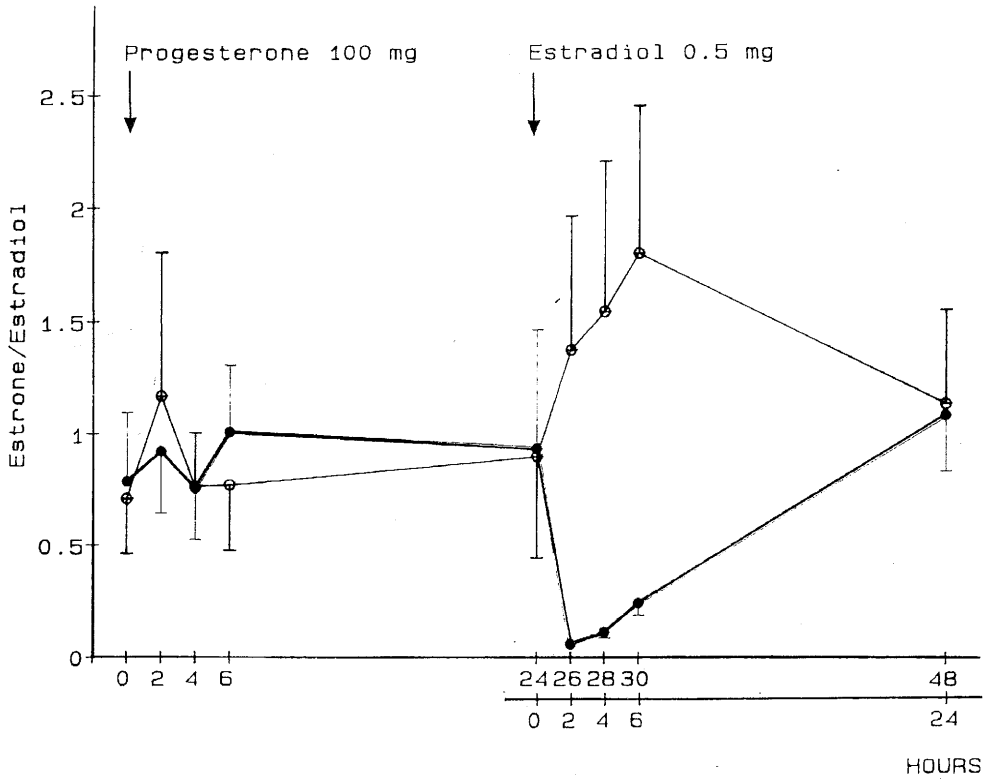

Fig. 7. Estrone to estradiol plasma levels (mean ± S.E.M.) after vaginal (●) and oral (○) administration of estradiol.

Plasma estrogen sulfates (Figs. 8 and 9). After oral E_2 administration, both estrogen sulfate levels increased but the peak was not evidenced at the same time (Table IV). In fact, the mean peak level of E_2S (522 ± 245 pg/ml) was observed at $0E_2$ + 6 h (Fig. 8) while that of E_1S (3046 ± 542 pg/ml) occurred earlier at $0E_2$ + 4 h (Fig. 9).

After vaginal administration, the peaks of the two sulfates occurred concomitantly at $0E_2$ + 6 h (Figs. 8 and 9) reaching a mean value of 816 ± 432 pg/ml and 3603 ± 718 pg/ml for E_2S and E_1S respectively (Table V). Then there was a gradual decrease, but the mean level at $0E_2$ + 24 h was higher than the baseline level for both sulfates. It is noteworthy that E_1S levels were always higher than those of E_2S, whatever the administration mode (Table V).

The comparison between the two ways of administration has evidenced that the levels of E_1S were rather similar whereas those of E_2S were notably higher when E_2 was administered via the vagina.

Discussion

Progesterone administration

After oral administration, the fact that P levels peaked at 0P + 2 h in all subjects agrees with the findings of Morville et al. [10] but is at variance with our previous

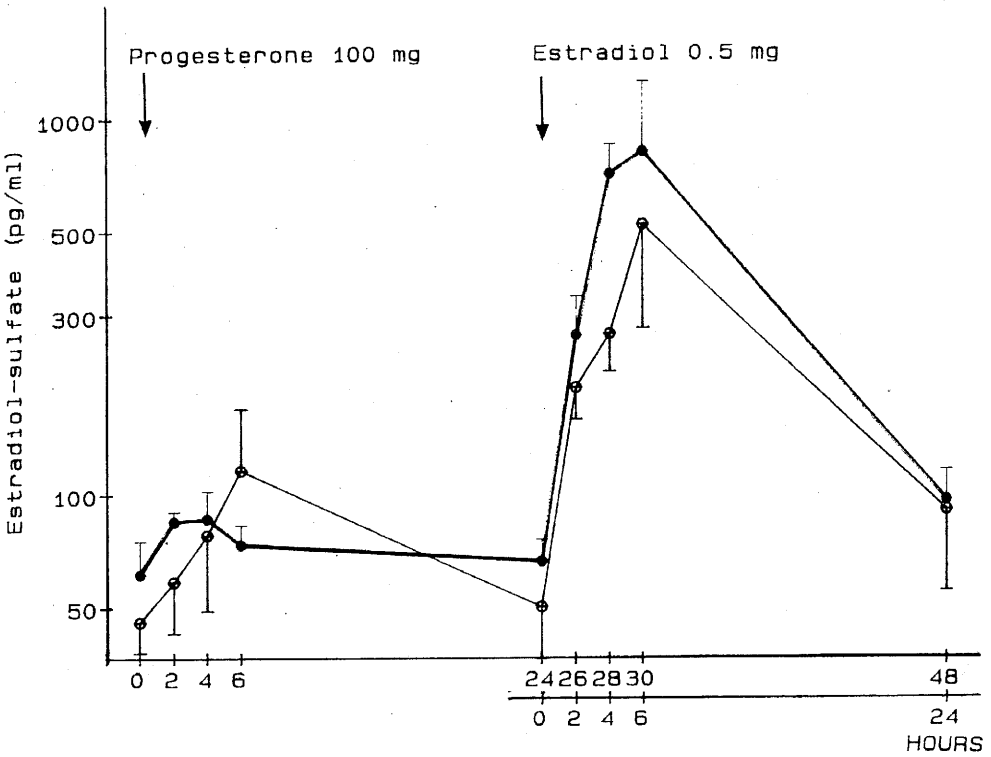

Fig. 8. Plasma levels (mean ± S.E.M.) of estradiol sulfate after vaginal (●) and oral (○) administration of estradiol.

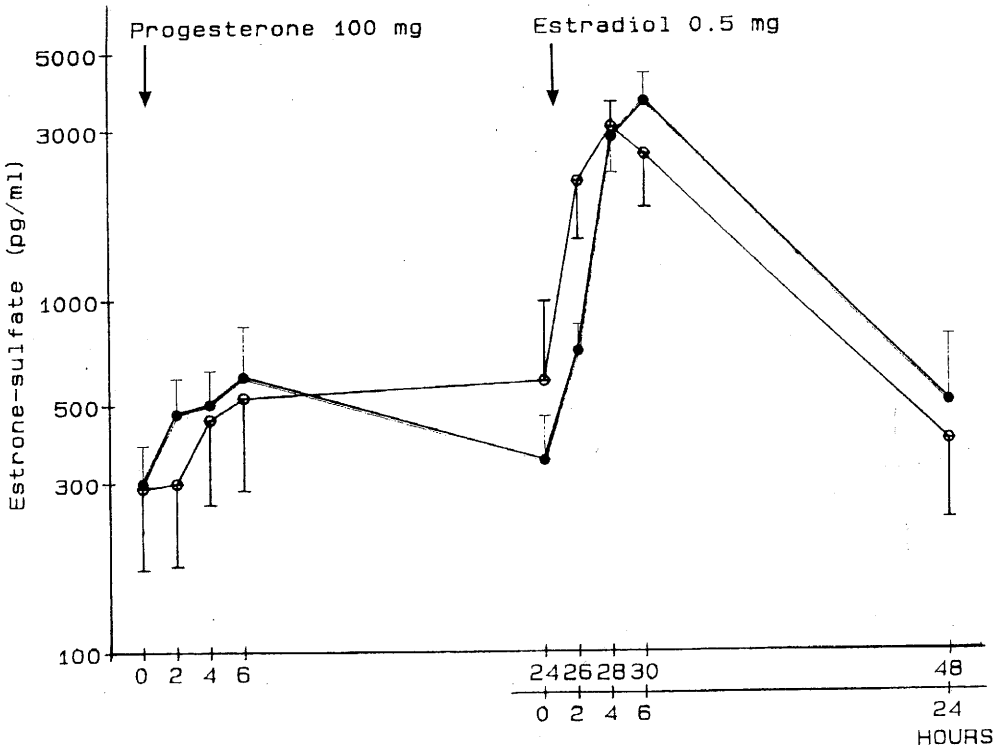

Fig. 9. Plasma levels (mean ± S.E.M.) of estrone sulfate after vaginal (●) and oral (○) administration of estradiol.

TABLE IV

PLASMA STEROID SULFATE LEVELS (MEAN AND (RANGE)) AFTER VAGINAL ADMINISTRATION OF PROGESTERONE ($t = 0$) AND ORAL ADMINISTRATION OF ESTRADIOL ($t = 24$ h).

Time (h)	E1S (pg/ml)	E2S (pg/ml)	DOCS (pg/ml)
0P	289	46	63
(P vaginal route)	(102–740)	(27–73)	(28–115)
2	298	59	78
	(85–836)	(16–110)	(23–112)
4	455	78	61
	(100–1160)	(15–206)	(24–82)
6	524	116	64
	(91–1560)	(13–346)	(12–109)
24(0E2)	584	50	77
(E2 oral route)	(77–2563)	(15–99)	(35–148)
26	2143*	193*	81
	(309–4087)	(89–321)	(48–174)
28	3046*	268*	133
	(648–4275)	(150–469)	(56–256)
30	2551*	522*	121
	(779–5441)	(113–1562)	(44–287)
48	392	91	74
	(106–1097)	(31–262)	(26–206)

*$P < 0.01$.

TABLE V

PLASMA STEROID SULFATE LEVELS (MEAN AND (RANGE)) AFTER ORAL ADMINISTRATION OF PROGESTERONE ($t = 0$) AND VAGINAL ADMINISTRATION OF ESTRADIOL ($t = 24$ h).

Time (h)	E1S (pg/ml)	E2S (pg/ml)	DOCS (pg/ml)
0P	298	61	102
(P oral route)	(110–686)	(31–122)	(33–176)
2	472	85 267*	
	(125–786)	(73–109)	(180–398)
4	502	87	207**
	(194–1022)	(26–143)	(95–394)
6	601	74	114
	(254–1762)	(42–105)	(54–192)
24(0E2)	345	67	120
(E2 vaginal route)	(134–875)	(42–108)	(30–192)
26	708**	266**	104
	(332–1117)	(95–463)	(25–194)
28	2842*	712*	175**
	(983–4215)	(387–1296)	(42–290)
30	3603*	816	117
	(2142–5811)	(85–2937)	(35–179)
48	504	97	136
	(62–1852)	(50–177)	(45–343)

*$P < 0.05$. $P < 0.05$.

results [4] as well as with other data [11–15], where the peak occurred at variable times. These differences might be the consequence of variations in absorption rate and bioavailability which were shown to be related to particle size and vehicle [16]. Eating habits and times at which meals are taken may probably also have an influence as demonstrated for many drugs [17]. The latter factor has been strictly controlled in the present study since food intake and the meal timing were standardized.

Regarding the P peak-level, high inter-individual variability was observed, a finding consistent with our previous results [4] and other data [10–15]. This might be related to the variability of the absorption and/or the metabolic rates. In any case, the mean concentration at the peak did not exceed 1.5 ng/ml, a value which was lower than all the data reported for similar administered doses. In fact, it has already been shown that specific techniques should be used to determine plasma levels of P after its oral administration, since P metabolites, which are produced in important amounts, may interfere in the RIA [4]. This is particularly true when P was analyzed by RIA either directly [12,13,15,16] or after inadequate chromatographic purification [10,11,14].

After vaginal administration, the pattern of mean P levels showed a rapid rise followed by a gradual decrease, which was comparable to that reported by several authors [15,18–24]. However, though it was demonstrated that plasma levels were related to the vehicle used for vaginal administration [22], the mean peak level of the present series was lower than the data obtained with similar [15,18], or even lower doses [19,21,23]. The present data were comparable to those of Erny et al. [24] with the same dose and with those of Kalund-Jensen and Myrén [20] with a ten times lower dose. The limited specificity of the techniques used might be the cause of these discrepancies.

In any case, the vaginal administration provided higher plasma P levels than the oral route. In addition, the levels were sustained for a longer time; they were still higher at 0P + 24 h than baseline values, though the difference was not statistically significant. This is consistent with other data [15,18–24] and explains the fact that oral therapy with 300 mg of micronized P failed to provide normal endometrial secretion, while vaginal application of the same dose produced a secretory endometrium similar to that of natural cycles [25].

Progesterone metabolites

The conversion of orally or intramuscularly administered P to DOC was demonstrated by Ottosson et al. [12,26] and the present data are consistent with those findings. However, while it was reported that the peak occurred at variable times [11], here it was evidenced very rapidly at 0P + 2 h after oral administration.

After vaginal administration, a different pattern of DOC concentrations was found here for the first time. In fact, DOC increased in only three subjects and reached markedly lower levels than those attained by the oral route. This is similar to the lower DOC levels which are observed after intramuscular administration [11].

The higher DOC levels, generated by oral administration, suggest that the conversion of P to DOC probably occurs in the intestine, since no steroid 21-hydroxylase activity could be detected in human liver tissue [27,28]. Indeed this enzymatic activity has been demonstrated in the duodenum of a prepubertal boy [28].

The variations of DOC sulfate levels after P administration are reported here for

the first time. They displayed a pattern with two peaks whatever the way of P administration, but the levels attained were less important after the vaginal route. Concerning this particular pattern it is noteworthy that the second peak occurred at 0P + 28 h or at $0E_2$ + 4 h. This time coincides with E_2 and E_1 peaks so that it is plausible that estrogen increase could have stimulated 21-hydroxylation of P, as shown by MacDonald et al. [29] in the case of estrogen treatment of pregnant women with a dead fetus.

Deoxycorticosterone is a pure mineralocorticoid, but the implication of its biosynthesis from exogenous P in the hydro-electrolyte metabolism remains to be established, since P exerts anti-mineralocorticoid and anti-hypertensive effects [30].

The variations of 5α- and 5β-pregnanolone levels after oral P administration and the prevalence of the 5α-isomer are consistent with the data of Arafat et al. [31].

The patterns of the two pregnanolone levels after vaginal P administration are described here for the first time and they show that the 5α-isomer is always prevalent. In any case, the levels were markedly lower than after P ingestion.

In view of these findings, while the 5β-reduction of P is accomplished almost exclusively in the liver [32], it may be suggested that oral P was converted to 5α-reduced metabolites in the intestine. Indeed, the latter type of conversion is predominant in canine [33,34] and human [35] small intestine. Moreover, low levels of 5α-pregnanolone after oral P administration were observed in a subject who had a Billroth II operation, which allowed oral P to pass directly from the stomach to the jejunum, thus bypassing the duodenum [31].

In any case, whichever the route of P administration, the prevalence of the 5α-reduced metabolites, agrees with recent studies, which have shown that most of P metabolism (60–65%) is processed via 5α-reduction [32].

Estradiol administration

Unconjugated estrogens. Though the pattern of estrogen levels after E_2 vaginal administration was shown to be dependent on the particle size on the vehicle and the status of the vaginal mucosa [36] and to be different in pre- and post-menopausal women [20,23], the increase of E_2 prevails over that of E_1 [23,37,38] and this is consistent with our findings. Moreover, the prompt increase of E_2 to a rapid maximum, followed by a very gradual decline to a mean level observed at $0E_2$ + 24 h which is significantly higher than baseline value, agrees with results from other authors [20,23,37,38].

However, in comparison with literature data obtained with similar E_2 doses, some differences were observed concerning the time of peak occurrence [20,23,37,38] and the mean peak value [38]. These variations might be due to the vehicle used, to inter-individual variability and to differences in the specificity of the techniques used for plasma E_2 determination. Indeed, the cream vehicle [39] and the use of vaginal suppositories [20,23,36] appeared to retard the vaginal absorption of micronized E_2, while an aqueous suspension [37] had the opposite effect.

The predominance of E_1 over E_2 levels after E_2 oral administration agrees with liteature data [3] and is in contrast with the pattern after vaginal administration, which displays much higher E_2 plasma levels. It was thought that the major cause of the so-called first pass effect influencing the absorption of E_2 was metabolism in

the liver. However, when E_2 was administered in the portal vein of the dog, the fraction absorbed as E_2 was 90% [40]. In view of the similar conversion ratios of E_2 to E_1 for portal and systemic administration of E_2, Longcope et al. [40] have suggested that there was little metabolism by the liver itself. Thus the major portion of the first pass effect was actually due to intestinal rather than to hepatic metabolism.

Estrogen sulfates. Most reports dealing with the relationship between the estrogens administered through different routes and the subsequent estrogen profiles in plasma were restricted to the assay of unconjugated E_2 and E_1. Indeed estrogen sulfates have limited biological activity though E_1S might be considered as an estrogen reservoir [41,42] in view of its long half-life [43], its high levels in peripheral plasma [41,44,45] and its conversion to unconjugated E_1 [42,46]. Although, E_1S and E_2S were determined after oral E_2 administration [47,48] and total E_1 (mainly E_1S) was measured after vaginal [36] and oral or percutaneous [49] administration of E_2, it is difficult to compare the present data with those findings, since the administered dose and the analytical techniques are different.

In any event, both oral and vaginal administration of E_2 lead to similar high E_1S levels, which confirm a predominant hepatic production of this sulfoconjugate. This is corroborated by the lower levels of E_1S in patients with cirrhosis [50] and by the concentration gradient between the femoral artery and the hepatic vein [45].

The E_2S levels obtained after vaginal administration are reported here for the first time. Their pattern was very similar to that of E_1S, but their levels were always lower. It is noteworthy that the higher levels of E_2S, attained after vaginal administration, confirm the fact that the conversion of E_2 to E_1 occurs mainly in the intestine.

Conclusion

In conclusion, the comparison of the steroid profiles, after administration of P and E_2 through oral and vaginal routes, has evidenced rapid metabolism mainly in the intestine. Thus, P levels attained after oral administration were very low, well below the lower limit of the normal range observed in the luteal phase [51], though the administered dose was higher than the production rate of P during the luteal phase of the menstrual cycle [52]. It appears then that the vaginal route, generating notably higher plasma P levels, should be preferred. In view of the higher plasma E_2 levels attained after vaginal administration, this route appears more adequate. Adverse hepatic effects are associated with oral E_2 therapy, leading to an increase in the serum levels of hepatic proteins such as sex hormone-binding globulin (SHBG), thyroxine-binding globulin (TBG) and renin substrate which are all significantly elevated in peripheral plasma [53,54]. The clinical significance of these elevations remains however to be established [54].

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