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CELLULAR AND SUBCELLULAR METABOLISM OF PROGESTERONE BY THE HUMAN PROLIFERATIVE AND SECRETORY PHASE ENDOMETRIUM AND MYOMETRIUM

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SUMMARY

An investigation of the metabolism of [1,2-^{3}H]-progesterone by human proliferative and secretory phase uterine tissue and the subcellular localization and metabolism of progesterone showed that in the endometrium and the myometrium progesterone was mainly converted to 5α-pregnane-3,20-dione and 20α-hydroxy-4-pregnen-3-one. Smaller quantities of 20α-hydroxy-5α-pregnan-3-one, 6β-hydroxy-progesterone and unidentified polar metabolites were also formed. Qualitatively this metabolism did not appear to vary significantly in the human endometrium and myometrium. However, quantitative variations between the endometrium and myometrium were apparent in both the proliferative and secretory phases of the cycle. Conversion of progesterone to 5α-pregnane-3, 20-dione was higher in the endometrium than in the myometrium, while more of 20α-hydroxy-4-pregnen-3-one was formed by the myometrium. More 20α-hydroxy-4-pregnen-3-one was formed in the secretory than in the proliferative phase.

Localization of progesterone in the subcellular fractions of the endometrium and myometrium showed that progesterone and its metabolites are mainly localised in the cytosol fraction, wherein they bind specifically to receptor protein. The nuclear uptake was considerably lower than that of the cytosol fraction. The subcellular metabolism of progesterone in the individual fractions, in the presence of co-factors, revealed that the conversion of progesterone was higher in the cytosol fraction. The major metabolite formed in the nuclear fraction of endometrium and myometrium was 5α-pregnane-3,20-dione. In the mitochondrial fraction 5α-pregnane-3,20-dione, 20α-hydroxy-5α-pregnan-3-one and some highly polar compounds were formed. 6β-hydroxy-progesterone was formed to a considerable extent by the microsomal and mitochondrial fractions. In the cytosol fractions of both the endometrium and myometrium, 20α-hydroxy-4-pregnen-3-one was the major metabolite with small amounts of 6β-hydroxy-progesterone, 5α-pregnane-3, 20-dione and 20α-hydroxy-5α-pregnan-3-one.

INTRODUCTION

Steroid hormones are considered to act by selective localization in the hormone-sensitive organs. Early attempts failed to provide any evidence for the specific uptake of progesterone by the uterus or other target tissues [1]. Laumas and Farooq[2] showed that progesterone is retained in the rat uterus after an initial rapid disappearance. This was later confirmed by other workers [3–7]. Cellular and subcellular metabolism of progesterone in the rat uterus has been investigated extensively [8–12], but little information is available on metabolism of this steroid in the human uterus [13–17]. Progesterone was shown to be metabolized mainly to the 5α-reduced and 20α-reduced compounds in the human uterus [13–17]. It has been suggested that certain metabolites of steroid hormones play an important role in the action of the parent hormone [12, 18–21] and it has been proposed that progesterone acts by binding specifically to the human uterine cytosol fraction [22–26]. However, the metabolic fate of progesterone in human uterus and cellular fractions is not fully known.

The present study describes an investigation on the comparative metabolic potential of the human endometrium and myometrium during the proliferative and secretory phases of the menstrual cycle. The enzymes responsible for the metabolic conversions of progesterone at the cellular and subcellular levels have been studied. The molecular mechanism of action of progesterone has been discussed in relation to its metabolism.

EXPERIMENTAL

Radioactive steroid. [1,2-^{3}H]-progesterone (S.A. 47·8 Ci/mmol) was obtained from New England Nuclear Corp., Boston, Mass., U.S.A. and purified by t.l.c. using chloroform–acetone (9:1 v/v).

Tissue preparation. Normal uterine tissue was collected during hysterectomy operations from the All India Institute of Medical Sciences Hospital, New Delhi. The tissue was brought to the laboratory on ice and the endometrium was scraped from the myometrium under ice-cold conditions. Both tissues were

[illegible] over ice and incubated as described [illegible] fractionation was carried out by [illegible] centrifugation as described previously [illegible] modification.

[illegible] of the nuclear pellet. The 800 *g* nuclear [illegible] suspended in Tris–HCl buffer (pH 7·4, [illegible] homogenized in a Teflon homogenizer. [illegible] was gently layered on the top of [illegible] solution in the Tris–HCl buffer and [illegible] 000 *g* for 60 min. The nuclear pellet [illegible] the bottom was used for subsequent [illegible].

[illegible] The tissue minces in 25 ml capacity [illegible] were incubated at 37°C with [1,2-^{3}H]-[illegible] in Krebs–Ringer phosphate [illegible] for 15, 30, 60, 90 and 120 min. At [illegible] incubation, the contents of the flasks [illegible] centrifuged immediately in a refrigerated International Centrifuge Model PR-2 at 4°C. The supernatant was decanted and the tissue pellet was washed [illegible] centrifuged twice with cold buffer to remove [illegible] radioactivity. The tissue was homogenized [illegible]. The steroid was extracted from the [illegible] with acetone. An aliquot of the acetone [illegible] was transferred to counting vials and dried under [illegible] of nitrogen at 45°C.

[illegible] study: incubation. The minced tissue [illegible] of endometrium and about 500 mg of [illegible] or the subcellular fractions (prepared [illegible] endometrium or 1 g of myometrium) were [illegible] into 25 ml flasks containing [illegible]-progesterone and Krebs–Ringer phosphate [illegible] with 1 mg glucose/ml. The ratio of [illegible] was 1:10 (w/v). For studies on subcellular [illegible] each flask contained 22 μmol of glucose [illegible] mol of glucose-6-phosphate, 2·3 μmol [illegible] μmol of NADPH in the buffer. The [illegible] were carried out at 37°C with constant shaking [illegible] h for whole tissue and for 1 h for the subcellular fractions. The reaction was stopped by [illegible] 5 vol. of distilled acetone and the flasks were left at room temperature for 24 h with occasional shaking.

[illegible] The extraction of radioactive steroids [illegible] carried out as described by Pearlman *et al.* [28] [illegible] modification. The aq. extract was adjusted [illegible] with 1 N NaOH before solvent extraction [illegible] Lipids were removed as described by [illegible] The delipidized extract was dried under [illegible] nitrogen at 50°C and subjected to t.l.c. [illegible] After incubation and extraction the [illegible] steroids were separated by t.l.c. on silica [illegible] mm thickness) plates using the following [illegible]:

[illegible] ane–ethyl acetate (5:2 v/v)
[illegible] oroform–acetone (9:1 v/v)
[illegible] roform–methanol (99:1 v/v)
[illegible] ane–diethyl ether (8:2 v/v)
[illegible] ohexene–cyclohexanone (9:1 v/v)

and by paper chromatography on Whatman No. 2 chromatography paper using hexane formamide solvent system [30]. The radioactive steroids were located with a chromatogram scanner (Packard Model 7200) and quantitated in a liquid scintillation spectrometer. The mobility of the steroids was generally indicated by R_F. When the chromatograms were overrun, mobility of the steroids was indicated by R_s (relative to the mobility of progesterone). The identity of the steroid metabolites was confirmed by acetylation, saponification of acetylated compounds and oxidation as described earlier [21] and recrystallization to constant S.A. [31].

Radioactivity counting. Radioactivity was measured in a liquid scintillation spectrometer (Packard Tricarb Model 3314) using 10 ml of scintillation fluid (4 g of 2,5-diphenyloxazole and 0·1 g of 1,4 bis-2-(4-methyl-5-phenyloxazolyl)-benzene dissolved in 1 l of toluene).

RESULTS

The radioactive steroids, extracted from the endometrium and myometrium when chromatographed in system I, resolved into α, β, γ, δ, ε and χ peaks. Each peak was rechromatographed and identified as follows.

The α-peak. In the initial t.l.c. system I this peak had mobility identical with authentic 5α-pregnane-3,20-dione (R_s 1·57). The radioactive steroid in the α-peak was extracted and rechromatographed in System V, where 5α-pregnane-3,20-dione separated well from the 5β epimer. The former had an R_F of 0·51 and the latter 0·6. These two steroid epimers could not be separated when chromatographed in system II, III and IV. The major amount of the radioactive steroid had mobility (R_F 0·51) identical with 5α-pregnane-3,20-dione in system V. Both sample and the standard steroid could not be acetylated and oxidation produced no change in the mobility of the sample. Finally crystallization to constant S.A. of the sample confirmed the identified sample as 5α-pregnane-3,20-dione.

The β + γ peak. In the initial t.l.c. system I a single peak which had a mobility similar to that of progesterone was designated as $\beta + \gamma$ peak. This peak was resolved into β and γ peaks by further t.l.c. in system II. In this system β peak moved with authentic progesterone and γ peak with pregnanolone isomers. After acetylation and further chromatography the steroid in the β-peak had a mobility identical with that of progesterone (R_F 0·76). Recrystallization to constant S.A. confirmed that the radioactivity in this peak was due to progesterone.

The γ-peak moved with an R_F of 0·57 and was identical with 20α-hydroxy-5α-pregnan-3-one. This was rechromatographed in system III, in which it had the same mobility as the authentic sample.

The δ peak. The δ peak of the initial t.l.c. system I consisted mainly of 20α-hydroxy-4-pregnen-3-one. Rechromatography of this region on paper in hexane

formamide, indicated that it was 20α-hydroxy-4-pregnen-3-one. On acetylation, the acetate of the sample and the standard sample had identical mobility (R_F 0·5) in system I. Saponification of 20α-hydroxy-4-pregnen-3-one-20-acetate yielded a compound with R_F 0·42 in the hexane-formamide system identical with that of authentic standard 20α-hydroxy-4-pregnen-3-one. Oxidation of the sample with chromium trioxide yielded progesterone which was identified chromatographically. Recrystallization to constant S.A. confirmed it to be 20α-hydroxy-4-pregnen-3-one.

The ε peak. The ε peak in system I had a very low chromatographic mobility corresponding to authentic 6β-hydroxy-progesterone. Radioactive steroid in this peak was extracted and rechromatographed in system II when it moved with an R_F of 0·31; authentic pregnanediol isomers also had the same mobility. The radioactive steroid in this region was acetylated and chromatographed in system I where it moved with the acetate of 6β-hydroxy-progesterone with an R_F of 0·13. The R_F of authentic pregnanediol acetate was greater than 0·5 in this system. Saponification of the sample gave a product with the same mobility as 6β-hydroxy-progesterone. The compound was tentatively identified as 6β-hydroxy-progesterone.

The χ peak. The χ peak consisted of the radioactivity starting from the origin up to the base of the ε peak. It constituted the highly polar compounds. On rechromatography in system I, the radioactivity again remained near the origin suggesting the high polarity of the metabolite(s). This region was not investigated further.

Metabolism by the proliferative endometrium. After 2 h of incubation, the endometrial tissue (300 mg) in the proliferative phase converted, on an average, 26·2% of progesterone to its metabolites (Table 1 and Fig. 1). Amongst the identified metabolites 20α-hydroxy-4-pregnen-3-one was the major metabolite, being 7·1% of the total metabolites. 5α-pregnane-3-20-dione constituted 4·7% of the metabolites. Formation of the pregnanolone isomers was not very fast, since, even after 2 h of incubation, only 2–3% of 20α-hydroxy-5α-pregnan-3-one was formed. Polar metabolites constituted about 7·6% of the total metabolites. Untransformed progesterone constituted 73·8%.

Table 1. Metabolism of progesterone in the human endometrium

Steroids	Proliferative phase	Secretory phase
5α-pregnane-3,20-dione	4.7	2.7
progesterone	73.8	79.2
20α-hydroxy-5α-pregnan-3-one	2.3	1.1
20α-hydroxy-4-pregnen-3-one	7.1	6.8
6β-hydroxy-progesterone	1.7	0.9
polar compounds	7.6	7.9

Results are expressed as per cent of the total steroid per 300 mg of endometrial tissue. The values are mean of three experiments. 41.8 picomol were used for each incubation and the incubations were continued for 2 h at 37°C.

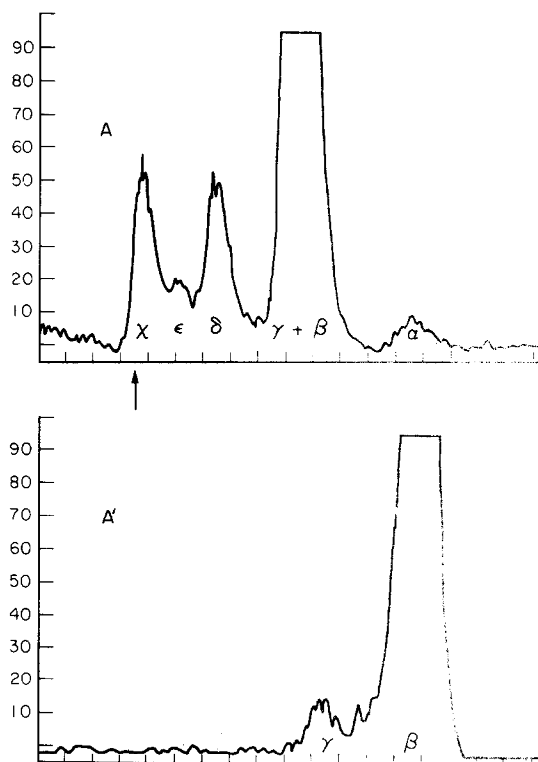

Fig. 1. Representative radiochromatogram scan of the metabolism of [1,2-^{3}H]-progesterone in the human endometrium.

A: Chromatogram developed in hexane:ethyl acetate (5:2) representing α = 5α-pregnane-3,20-dione, β + γ = progesterone + 20α-hydroxy-5α-pregnan-3-one, δ = 20α-hydroxy-4-pregnen-3-one, ϵ = 6β-hydroxy-progesterone and χ = polar compounds.

A′: β + γ peak rechromatographed in chloroform:acetone (9:1) to resolve into β = progesterone and γ = 20α-hydroxy-5α-pregnan-3-one

Metabolism by the secretory endometrium. In the secretory endometrium metabolic products were qualitatively similar to proliferative phase, but quantitative differences were apparent (Table 1). The total amount of progesterone converted was about 1·2 times more in the proliferative than in the secretory phase. Secretory phase endometrium converted less progesterone to 5α-pregnane-3,20-dione than in the proliferative phase, but more 20α-hydroxy-4-pregnen-3-one was formed in the secretory than in the proliferative phase. The pregnanolone isomer was formed to only a small extent. 6β-Hydroxy-progesterone was formed to a greater extent by the proliferative phase tissue while polar compounds accumulated more in the secretory phase tissue.

Metabolism of progesterone by the human myometrium in the proliferative and secretory phases. The metabolism of progesterone in the human myometrium is shown in Fig. 2. The major metabolites of progesterone formed in the myometrium were qualitatively similar to those in the endometrium. Table

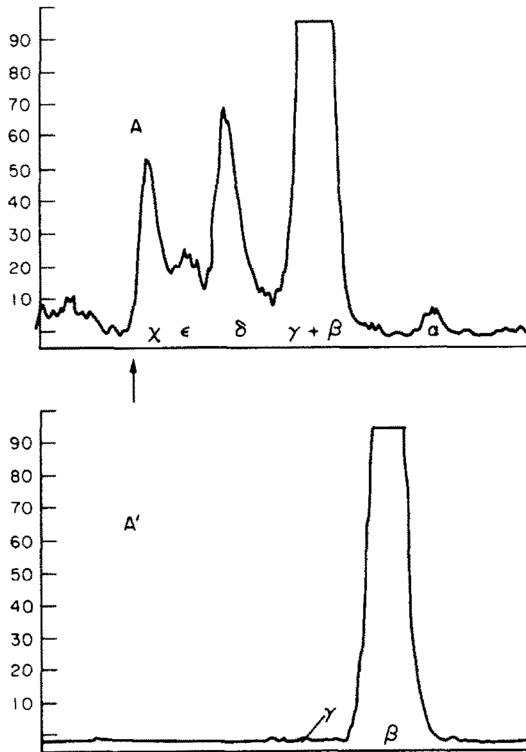

Fig. 2. Representative radiochromatogram scan of progesterone metabolism in the human myometrium. Peaks $\alpha,\beta,\gamma,\delta,\epsilon$, and χ represent steroids as in Fig. 1.

2 shows the amount of metabolites formed during the proliferative and secretory phases of the cycle. About 15·1% and 12·4% of progesterone was converted to its metabolites in the proliferative and secretory phase respectively. Conversion of progesterone by the proliferative myometrial tissue, was about 1·2 times higher than in the secretory phase.

Comparison of the metabolic potential of endometrium with that of myometrium in the two phases revealed that on unit weight basis, endometrium converted about 2·9 times more progesterone in the proliferative and about 2·7 times more in the secretory phases than the myometrium. In the myometrium 20α-hydroxy-4-pregnen-3-one was formed more than in the endometrium (Table 3) while less of 5α-pregnane-3,20-dione was formed in the myometrium when compared to the endometrium.

Subcellular uptake of progesterone. To investigate the fate of progesterone at the subcellular level, the localization of progesterone in different cell fractions of endometrium and myometrium was studied. Table 4 shows that 45·9% of progesterone and its metabolites were present in 105,000 *g* cytosol fraction after 90 min of incubation. The nuclear fraction contained about 37·4% of the total steroid. The mitochondrial and microsomal fractions contained nearly 16% of progesterone and its metabolites.

Subcellular distribution of [1,2-^{3}H]-progesterone and its metabolites in the myometrium was studied at different time intervals (Table 4). The 105,000 *g* cytosol fraction contained 44·3% of the total radioactivity at 15 min. At 120 min the uptake increased to 56·5%. The mitochondrial and the microsomal fractions contained rather low amounts of progesterone and its metabolites. The nuclear fraction contained about 37% of the steroid at 15 min and 22% at 120 min. In general the cytosol fractions contained more progesterone and its metabolites than the other subcellular fractions.

Subcellular metabolism of progesterone by the endometrium. The metabolism of progesterone by the nuclear fraction showed that on an average 13·6% of progesterone was converted (Table 5). The major metabolite formed was 5α-pregnane-3,20-dione and it constituted about 4·5% of the metabolites which is about 33·0% of the products formed (Fig. 3). Other metabolites like 20α-hydroxy-5α-pregnan-3-one, 20α-hydroxy-4-pregnen-3-one and 6β-hydroxy-progesterone were formed in considerably lower amounts.

In the mitochondrial fraction about 15·4% of the incubated progesterone was converted to its metabolites. Small amounts of 5α-pregnane-3,20-dione, 20α-hydroxy-5α-pregnan-3-one, 20α-hydroxy-4-pregnen-3-one and 6β-hydroxy-progesterone were found. The polar compound constituted a considerable amount of the metabolized progesterone. A similar picture was obtained from the microsomal fraction. In this fraction about 17·6% of progesterone was converted to its metabolites. Polar compounds constituted the major amount of biotransformed steroids. 6β-Hydroxy-progesterone was formed considerably in the microsomal fraction and made up about 14·2% of the products formed.

Among the subcellular fractions the cytosol fraction converted the maximum amount of progesterone. On an average 20·8% of progesterone was converted to its metabolites. In the cytosol fraction 20α-hydroxy-4-pregnen-3-one was the major metabolite, constituting about 10·7% of the total metabolites and it was 49·0% of the products formed (Fig. 3). In the cytosol fraction small amounts of 5α-pregnane-3,20-dione and 20α-hydroxy-5α-pregnan-3-one. 6β-hydroxy-progesterone and the polar compounds were also found.

Table 2. Metabolism of progesterone in the human myometrium

Steroids	Proliferative Phase	Secretory Phase
5α-pregnane-3,20-dione	1.4	0.6
progesterone	84.9	87.6
20α-hydroxy-5α-pregnan-3-one	0.5	0.2
20α-hydroxy-4-pregnen-3-one	6.5	5.3
6β-hydroxy-progesterone	1.1	0.7
polar compounds	3.0	4.8

Results are expressed as per cent of the total steroid per 500 mg of myometrial tissue. The values are mean of 3 observations. Amount of the steroid used and the incubation time as in Table 1.

Table 3. Comparative metabolism of progesterone in human endometrium and myometrium in the proliferative and secretory phases

Metabolites Formed	Endometrium		Myometrium	
	Proliferative	Secretory	Proliferative	Secretory
5α-pregnane-3,20-dione	17.9	12.9	9.3	4.8
20α-hydroxy-5α-pregnan-3-one	8.7	5.2	3.3	1.6
20α-hydroxy-4-pregnen-3-one	27.0	32.6	41.7	42.7
6β-hydroxy-progesterone	6.4	4.3	7.2	5.6
polar compounds	29.0	37.9	19.8	38.4

The results are expressed as per cent of the products formed.

Subcellular metabolism of progesterone by myometrium. The pattern of progesteone metabolism by the subcellular fractions of myometrium was similar to that of endometrium. In the nuclear fraction on an average 12·9% of progesterone was metabolized. As in the endometrium, 5α-pregnane-3,20-dione was the major metabolite in the nuclear fraction constituting about 3·8% of the metabolites and 29·4% of the products formed (Fig. 3). However, it was less than that in the endometrium. In the cytosol fraction 20α-hydroxy-4-pregnen-3-one was the major metabolite being about 61·2% of the products formed. More 20α-hydroxy-4-pregnen-3-one was formed by the myometrial cytosol fraction than by the endometrial fraction.

DISCUSSION

The results presented on the localization and metabolism of progesterone in the human endometrium and myometrium in their proliferative and secretory phases showed that in both the phases endometrium had a higher metabolic potential than myometrium. Further, the proliferative phase endometrium and myometrium were able to metabolize higher amounts of progesterone than their corresponding secretory phase tissues.

Table 4. Uptake of [1,2-^{3}H]-progesterone and its metabolites in subcellular fractions of the human endometrium and myometrium

Tissue	Time of incubation in minutes	Per cent of progesterone and its metabolites			
		Nuclear 800 *g*	Mitochondrial 10,000 *g*	Microsomal 105,000 *g*	Cytosol 105,000 *g*
Endometrium	90	37.4 ± 2.3	8.9 ± 3.1	7.6 ± 2.4	45.9 ± 2.4
Myometrium	15	36.9 ± 3.2	2.0 ± 1.0	16.3 ± 1.6	44.3 ± 4.0
Myometrium	30	22.2 ± 2.8	2.5 ± 0.8	6.9 ± 1.0	68.2 ± 8.0
Myometrium	60	25.0 ± 4.1	1.5 ± 0.8	11.1 ± 2.6	62.2 ± 6.5
Myometrium	120	22.6 ± 2.6	8.5 ± 1.3	12.3 ± 1.6	56.5 ± 3.8

Results are expressed as the mean ± S.D.

Table 5. Subcellular metabolism of progesterone by human endometrium

Steroids	Subcellular fractions			
	Nuclear 800 *g*	Mitochondrial 10,000 *g*	Microsomal 105,000 *g*	Cytosol 105,000 *g*
5α-pregnane-3,20-dione	4.5	1.1	1.6	0.2
progesterone	86.4	84.6	82.4	79.2
20α-hydroxy-5α-pregnan-3-one	1.2	2.5	1.5	2.1
20α-hydroxy-4-pregnen-3-one	0.8	1.2	2.2	10.7
6β-hydroxy-progesterone	0.3	2.3	2.5	2.1
polar compounds	3.5	5.0	7.5	2.6

Endometrial tissue (300 mg) was fractionated into nuclear, mitochondrial, microsomal and cytosol fractions. Each incubation was carried out in the presence of glucose 33 μmol, glucose-6-phosphate 11 μmol, ATP 2·3 μmol, NADPH 1·3 μmol and [1,2-^{3}H]-progesterone 20·9 picomol for 60 min. The data is the mean of three experimental values.

Table 6. Subcellular metabolism of progesterone in the human myometrium

Steroids	Subcellular fractions			
	Nuclear 800 *g*	Mitochondrial 10,000 *g*	Microsomal 105,000 *g*	Cytosol 105,000 *g*
5α-pregnane-3,20-dione	3.8	0.4	1.8	0.5
progesterone	87.1	83.9	80.1	76.8
20α-hydroxy-5α-pregnan-3-one	2.8	1.6	1.0	2.3
20α-hydroxy-4-pregnen-3-one	0.6	3.1	3.4	14.2
6β-hydroxy-progesterone	0.1	1.5	2.8	1.8
polar compounds	2.5	6.2	8.0	2.4

Myometrial tissue fractions were prepared and processed as described under Experimental and Table 5. The results represent mean values of three observations.

5α-Pregnane-3,20-dione was formed in larger amounts by the endometrial as compared with the myometrial tissue. Its formation was higher in the proliferative phase than in the secretory phase both by the endometrial as well as the myometrial tissues. The results suggest that the enzyme 5α-reductase which converted progesterone to 5α-pregnane-3,20-dione was either activated or present in higher amounts in the proliferative phase. This suggests that the saturation of the C-4–C-5 double bond of progesterone and consequent formation of 5α-pregnane-3,20-dione may be an estrogen dependent step [32, 33]. A higher activity of the human proliferative uterine tissue to metabolize estradiol [20] and norethynodrel [21] supported the concept that estrogens in proliferative phase influence the metabolic activity of the uterus. The concentration of 20α-hydroxy-4-pregnen-3-one showed a different picture. More of it was formed by the secretory phase uterine tissue as compared with the proliferative tissue. A higher concentration of 20α-hydroxy-steroid dehydrogenase in the secretory as compared to the proliferative phase has recently been reported [34].

The localisation of progesterone in the endometrial and myometrial cell fractions, clearly showed a preferential concentration in the cytosol fraction and next in the nucleus with very low amounts in the mitochondria and microsomes. The two major progesterone metabolites 5α-pregnane-3,20-dione and 20α-hydroxy-4-pregnen-3-one were formed mainly in the nucleus and cytosol fraction. In the mitochondria and microsomes, 5α-pregnane-3,20-dione, 20α-hydroxy-5α-pregnan-3-one, 6β-hydroxy-progesterone and some highly polar compounds were formed.

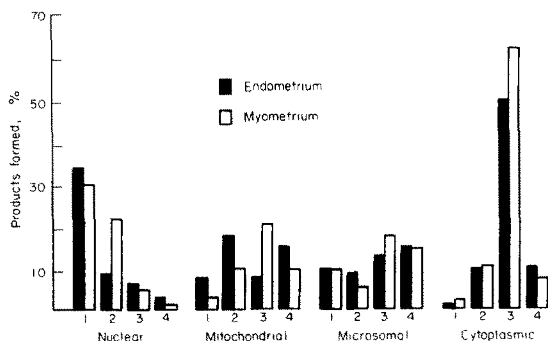

Fig. 3. Subcellular metabolism of progesterone in the nuclear, mitochondrial, microsomal and cytosol fractions of human endometrium ■ and myometrium □.
1: 5α-pregnane-3,20-dione, 2: 20α-hydroxy-5α-pregnan-3-one, 3: 20α-hydroxy-4-pregnen-3-one and 4: 6β-hydroxy-progesterone.

The cytosol fractions of both endometrium and myometrium converted major amounts of progesterone when calculated on a per cent conversion basis. However, if one considers the number of enzymes involved in the metabolism of progesterone, the mitochondrial and the microsomal fractions, which contain less protein, might convert relatively more progesterone than nuclear and cytosol fractions in terms of unit protein. The total concentration of metabolites formed by the mitochondrial and microsomal fractions may not contribute much, since total amount of steroid taken up by these fractions is very low as compared with the cytosol and nuclear fractions.

A higher concentration of 5α-pregnane-3,20-dione in uterine nuclei suggested the nuclear localization of the enzyme 5α-reductase. The nuclear concentration of this enzyme was demonstrated in rabbit myometrium and hypothalamic nuclei [32] and in the rat uterine and hypothalamic nuclei [8, 35]. Further significance of the nuclear localization of 5α-pregnane-3,20-dione is indicated by its high ability to compete with progesterone for its receptor [22, 23]. This suggests that different concentrations of 5α-pregnane-3,20-dione formed during different phases of the cycle may be responsible for the manifestation of progesterone action. Like 5α-pregnane-3,20-dione in the uterine nuclei, dihydrotestosterone, a biologically more potent metabolite than the parent compound testosterone forms in the prostate nuclei [36].

The other major metabolite, 20α-hydroxy-4-pregnen-3-one in the human endometrium and myometrium was localized in the cytosol fraction. The presence of the enzyme 20α-hydroxy steroid dehydrogenase in the cytosol fraction has been shown in the rabbit myometrium and hypothalamus [32] and in the

rat uterus and pituitary [8, 33]. 5α-Pregnane-3,20-dione appears to decline in favour of 20α-hydroxy-4-pregnen-3-one in the post ovulatory uterine tissue. It is not clear what specific progestational event is facilitated by this alteration.

The higher concentration of progesterone has been suggested to inactivate its own receptors [23, 37], while estradiol facilitates the progestrone receptor synthesis [38]. In the proliferative phase progesterone receptors may be protected from degradation or inactivation by faster metabolism of progesterone at the site of action and also by the higher conversion of progesterone to 5α-pregnane-3,20-dione. Thus 5α-pregnane-3,20-dione, although not very active in the bioassays for progesterone [39] and in maintaining pregnancy [40], may have a different role in the mechanism of progesterone action than progesterone *per se*. The nuclear formation of 5α-pregnane-3,20-dione suggested that the action of progesterone at the nuclear level may probably be modulated by the competition of 5α-pregnane-3,20-dione for the nuclear receptors. In the secretory phase, however, a different picture may prevail. Along with progesterone, a higher concentration of 20α-hydroxy-4-pregnen-3-one may also decrease the progesterone receptor concentration which may be the action of 20α-hydroxy-4-pregnen-3-one. An increase in the 20α-hydroxy-steroid dehydrogenase enzyme in the rat uterus has also been reported from day 11 to day 22 of pregnancy [41] when progesterone receptor concentration is decreasing [42].

Evidence presented in this communication suggests that the metabolism of progesterone in the uterine tissue is an important event in the mechanism of its action and the metabolites of progesterone may bear a specific role in the sequence of the physiological events at the molecular level. The different metabolic potential of endometrium and myometrium in the two phases of the cycle is of special significance in maintaining the required hormone milieu in the hormone sensitive tissue. The metabolism of progesterone may thus be a local regulator of the molecular events initiated by progesterone for the steroid to exert its action.

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