Effect of Oestradiol-17 β and Progesterone on the Metabolism of [1,2-³H]Progesterone by the Rabbit Endometrium and Myometrium

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The metabolism of [³H]progesterone in the rabbit endometrium and myometrium was studied *in vitro*. The major metabolites identified were 5 α -pregnane-3,20-dione, 20 α -hydroxypregn-4-en-3-one, 3 β -hydroxy-5 α -pregnan-20-one and 5 α -pregnane-3 β ,20 α -diol. Other minor metabolites tentatively identified were 3 α -hydroxy-5 β -pregnan-20-one, 20 α -hydroxy-5 β -pregnan-3-one and 5 β -pregnane-3 α ,20 α -diol. The ability of the endometrium to metabolize progesterone on a unit weight basis was about 2.7 times that of the myometrium. The metabolism of [³H]progesterone in the rabbit uterus under the influence of oestradiol-17 β and progesterone was studied. The ability of the oestradiol-treated rabbit uterus to metabolize progesterone was increased to 3.47 times that of the ovariectomized control uterus, whereas the oestradiol-primed progesterone-treated rabbit uterus metabolized only 1.86 times that of the control. Study of the metabolism of progesterone with uterine subcellular preparations revealed that the 5 α -reductase enzyme was present mainly in the nuclear fraction; 20 α -hydroxysteroid dehydrogenase was found in the cytosol fraction and 3 β -hydroxysteroid dehydrogenase in the particulate fraction of the uterus. The metabolic pathways of progesterone in the rabbit uterine tissue are discussed.

Selective localization of biologically active steroids in responsive organs has been demonstrated for oestradiol (Jensen & Jacobson, 1962), progesterone (Lawson & Pearlman, 1964; Laumas & Farooq, 1966; Laumas, 1967, 1969; Falk & Bardin, 1970), 5 α -dihydrotestosterone (17 β -hydroxy-5 α -androstane-3-one) (Bruchovsky & Wilson, 1968; Roy & Laumas, 1969) and corticosteroids (Beato *et al.*, 1970). Binding to the receptors has been suggested as the mechanism of progesterone action (Wiest & Rao, 1971; Verma & Laumas, 1973; King & Mainwaring, 1974) and oestrogen priming has been demonstrated to be essential for the manifestation of progesterone action (Ingle, 1954). However, little is known about the biochemical basis underlying oestrogen dependency on progestational response. Wichmann (1967) showed that progesterone is metabolized in the pregnant rat myometrium. In the rabbit uterus, conversion of progesterone into 20 α -hydroxypregn-4-en-3-one has been reported (Macartney & Thomas, 1969), but its precise metabolism and the effect of oestrogen and progesterone on the conversion of progesterone have not been investigated.

The present investigation was carried out to study the ability of rabbit endometrium and myometrium to metabolize progesterone under varied hormone conditions. A time-study was undertaken of the pattern of progesterone metabolism in the uterus.

The study reports on the effect of oestrogen and progesterone treatment on progesterone metabolism. The action of some subcellular preparations on progesterone metabolism was studied to reveal the localization of the enzymes responsible for the biotransformation of progesterone in the uterus. The molecular mechanism of the action of progesterone has been discussed in relation to its metabolism in the target organ.

Materials and Methods

Animals

Normal adult female rabbits of the All India Institute of Medical Sciences animal colony were used for the study of progesterone metabolism. To study the effect of oestradiol and progesterone on progesterone metabolism, the animals were ovariectomized and were divided into three groups of four animals each. The oestrogen- and progesterone-treatment groups were injected subcutaneously with 10 μ g of oestradiol-17 β in 0.5 ml of olive oil per day for 1 week. After this oestrogen treatment one of the two groups received 5 mg of progesterone per day subcutaneously for 7 days. Controls (ovariectomized animals) received the vehicle alone. The animals were killed 24 h after the last injection.

Radioactive steroid

[1,2-³H]Progesterone (specific radioactivity 47.8 Ci/mmol) was from New England Nuclear Corp. (Boston, MA, U.S.A.) The purity of the steroid was checked every 2 months by t.l.c. with chloroform/acetone (9:1, v/v) as the solvent.

Tissue preparation

Immediately after killing the animals the uterine horns were removed and placed in a clean petri dish, which was kept on ice. The horns were cut open longitudinally and the endometrium was gently scraped out from the myometrium and the tissues were weighed immediately.

Subcellular fractionation

The tissues were homogenized in an all-glass homogenizer. The tissue homogenate was filtered through a double layer of cheesecloth. This was then fractionated into nuclear (15 min × 800g), mitochondria (30 min × 10000g), microsomal (60 min × 105000g) and cytosol (105000g supernatant) fractions.

Purification of the nuclear pellet

The 800g nuclear pellet obtained from the uterine tissue was suspended in 0.2 ml of 0.01 M-Tris/HCl, pH 7.4, and homogenized in a Teflon homogenizer. The homogenate was layered on the top of 2 M-sucrose (in the same buffer) and centrifuged at 105000g for 60 min. The supernatant was discarded and the clear nuclear fraction was used for the metabolic study.

Incubation

[1,2-³H]Progesterone (41.8 pmol) was added to 25 ml incubation flasks and evaporated to dryness under a stream of N₂. Whole tissue minces (300 mg of endometrium and 500 mg of myometrium) were transferred to incubation flasks. Krebs-Ringer phosphate buffer (Cohen, 1959), pH 7.4, with 1 mg of glucose/ml was added in a ratio of 1:10 (w/v). The incubation was carried on at 37°C under constant shaking for 2 h. The reaction was stopped by addition of 4–5 vol. of distilled acetone and the mixture left at room temperature.

Subcellular fractions of the myometrial tissue (1 g) were prepared as described above. Each fraction was incubated for 1 h with 20.9 pmol of [1,2-³H]progesterone in Krebs-Ringer phosphate buffer containing 33 μmol of glucose, 11 μmol of glucose 6-phosphate, 2.3 μmol of ATP and 1.3 μmol of NADPH. The reaction was stopped and treated as above.

Extraction of steroids

The extraction of progesterone and its metabolites was carried out as described by Pearlman *et al.*

(1969), with slight modification. The aqueous extract was adjusted to pH 10 by the addition of 1 M-NaOH. Lipids were removed as described by Wiest (1963a). The extract was dried under N₂ at 45°C and chromatographed.

Analysis of the steroids

Thin-layer silica gel HF (E. Merck A.-G., Darmstadt, Germany) plates (0.25 mm thick) were activated at 110°C for 1 h. The samples and the authentic steroids were spotted on the plates and developed in the following solvent systems: I, hexane/ethyl acetate (5:2, v/v); II, chloroform/acetone (9:1, v/v); III, chloroform/methanol (99:1, v/v); IV, hexane/diethyl ether (4:1, v/v); V, cyclohexene/cyclohexanone (9:1, v/v). The steroids were located with u.v. light (254 nm) and by exposure to iodine vapour. The radioactive steroids were located by a radiochromatogram scanner (Packard model 7200). Descending paper chromatography was carried out on Whatman no. 2 paper by using the Zaffaroni hexane/formamide solvent system (Dominguez, 1967).

Acetylation, saponification, oxidation and reduction were carried out as described previously (Murugesan *et al.*, 1973). Crystallization to constant specific radioactivity was carried out by the method of Axelrod *et al.* (1965).

The radioactive steroids extracted from the endometrium and myometrium were initially separated by t.l.c. (system I). The chromatogram was separated into four regions, which were designated as α, β + γ, δ and χ. The steroids in these regions were characterized by repeated chromatography in different t.l.c. systems as described below and further analysed by microchemical reactions such as oxidation, reduction, acetylation and saponification and by recrystallization to constant specific radioactivity.

The α fraction contained mainly 5α-pregnane-3,20-dione and 5β-pregnane-3,20-dione. The steroids in this fraction were rechromatographed in systems II–V. 5α-Pregnane-3,20-dione and 5β-pregnane-3,20-dione were separated only in t.l.c. system V where they moved with *R_F* values of 0.51 and 0.60 respectively.

The initial chromatography (system I) did not separate the β and γ components, which were designated the β + γ fraction. It represented the region of progesterone and pregnanolone isomers. The steroids in this region were rechromatographed in t.l.c. system II, where progesterone [*R_F* 0.76 (β peak)], was separated from the pregnanolone isomers [*R_F* 0.55–0.56 (γ peak)].

The steroids in the γ fraction were rechromatographed in t.l.c. system III where 3β-hydroxy-5α-pregnan-20-one, 3α-hydroxy-5β-pregnan-20-one and 20α-hydroxy-5β-pregnan-3-one moved with *R_F* values of 0.49, 0.54 and 0.56 respectively. After acetylation

of the pregnanolone isomers and chromatography in t.l.c. system I, they moved with R_F values of 0.43, 0.47 and 0.36 respectively. Saponification and chromatography in t.l.c. system III yielded back 3 β -hydroxy-5 α -pregnan-20-one, 3 α -hydroxy-5 β -pregnan-20-one and 20 α -hydroxy-5 β -pregnan-3-one. Oxidation and chromatography of 3 β -hydroxy-5 α -pregnan-20-one yielded 5 α -pregnane-3,20-dione.

The δ peak represented the region where 20-hydroxypregn-4-en-3-one moved. The steroids in this region were rechromatographed on paper by using the Zaffaroni hexane/formamide system, where 20 α -hydroxypregn-4-en-3-one [R_X (relative to progesterone) 0.42] was separated from 20 β -hydroxypregn-4-en-3-one (R_X 0.74). The sample was acetylated and chromatographed in t.l.c. system I, where the acetylated sample had a mobility (R_F 0.5) identical with that of 20 α -acetoxypregn-4-en-3-one. Oxidation of the sample with chromium trioxide

yielded progesterone, which was identified chromatographically.

The χ peak (polar compounds) on the initial chromatogram (t.l.c. system I) represented the region starting from the origin of the chromatogram to the base of the 20-hydroxypregn-4-en-3-one region. The steroids in this peak were rechromatographed in t.l.c. system II, where they separated into three peaks. A highly polar peak at the origin, further analysis of which was not possible due to the lack of authentic standards. The second peak (R_F 0.32) co-chromatographed with the authentic pregnanediol isomers 5 α -pregnane-3 β ,20 α -diol and 5 α -pregnane-3 α ,20 α -diol. After acetylation and chromatography in t.l.c. system IV they separated with R_F values of 0.45 and 0.53 respectively. The acetylated sample had mobility (R_F 0.45) identical with the mobility of 5 α -pregnane-3 β ,20 α -diol. The third small radioactivity peak had a mobility identical with that of 5 β -pregnane-3 α ,20 α -diol (R_F 0.3). On acetylation and chromatography in t.l.c. system IV the diacetates of the sample and the authentic steroid 5 β -pregnane-3 α ,20 α -dioldiacetate had identical mobility (R_F 0.47). The steroids in the χ peak were thus identified tentatively by their R_F values.

The results were expressed (a) as the percentage of the substrate (where progesterone and its metabolites constituted 100%), and (b) as the percentage of the products formed (where metabolites constituted 100%).

Results

Metabolism of progesterone by rabbit endometrium

Fig. 1 and Table 1 show the pattern of progesterone metabolism by the rabbit endometrium. Four major radioactivity peaks were obtained, which were designated as α , $\beta + \gamma$, δ and χ (Fig. 1). The steroids in these peaks were characterized individually as described in the Materials and Methods Section. The total amount of progesterone converted by the endometrial tissue (300mg) after 2h of incubation was 58.4%, which constituted 19.5% per 100mg of tissue (Table 1). 5 α -Pregnane-3,20-dione accounted for 10.5% of the substrate, i.e. 17.9% of the products formed. In the endometrium 3 β -hydroxy-5 α -pregnan-20-one was formed as the major metabolite and was about 15.5% of the substrate and 26.5% of the products formed. Another major metabolite 20 α -hydroxypregn-4-en-3-one constituted 13.2% of the substrate and 22.6% of the products formed, 5 α -Pregnane-3 β ,20 α -diol accounted for 4.5% and the highly polar steroid, which did not move from the origin, constituted 10.5% of the substrate. 3 α -Hydroxy-5 β -pregnan-20-one, 20 α -hydroxy-5 β -pregnan-3-one and 5 β -pregnane-3 α ,20 α -diol, which were tentatively identified, formed 1.0, 0.4 and 1.1% respectively of the substrate.

Fig. 1. Radiochromatogram scan of the metabolism of progesterone by rabbit endometrial tissue

(a) Radiochromatogram scan of progesterone and its metabolites in the endometrium developed in the initial t.l.c. system [hexane/ethyl acetate (5:2, v/v)]. The radioactive peaks, α , $\beta + \gamma$, δ , and χ represent 5 α -pregnane-3,20-dione, progesterone + 3 β -hydroxy-5 α -pregnan-20-one, 20 α -hydroxypregn-4-en-3-one and polar metabolites respectively. (b) Rechromatography of the $\beta + \gamma$ fraction in chloroform/acetone (9:1, v/v) solvent system. The arrow indicates the origin of the chromatogram.

Table 1. *Metabolism of [1,2-³H]progesterone by the rabbit endometrium and myometrium*

The endometrial (300 mg) and myometrial (500 mg) preparations were incubated with [1,2-³H]progesterone (41.8 pmol) for 2 h at 37°C. The results are expressed as percentage progesterone converted and as percentage metabolites formed from the substrate. Values in parentheses are the percentage of the products formed. The results are the mean of five observations.

Steroids	Endometrial preparation	Myometrial preparation
5 α -Pregnane-3,20-dione	10.5 (17.9)	4.3 (11.9)
Progesterone	58.4 (19.5)*	36.0 (7.2)*
3 β -Hydroxy-5 α -pregnan-20-one	15.5 (26.5)	12.9 (35.8)
3 α -Hydroxy-5 β -pregnan-20-one	1.0 (1.7)	1.5 (4.2)
20 α -Hydroxy-5 β -pregnan-3-one	0.4 (0.7)	1.0 (2.8)
20 α -Hydroxypregn-4-en-3-one	13.2 (22.6)	5.2 (14.4)
5 α -Pregnane-3 β ,20 α -diol	4.5 (7.1)	1.7 (4.7)
5 β -Pregnane-3 α ,20 α -diol	1.1 (1.8)	0.4 (1.1)
Origin	10.5 (17.9)	7.5 (20.8)

* Percentage progesterone converted per 100 mg of endometrium and myometrium.

Table 2. *Crystallization of progesterone and its metabolites to constant specific radioactivity*

Progesterone and its metabolites, separated and identified by t.l.c. after incubation of [³H]progesterone *in vitro* with rabbit myometrium, were further recrystallized to constant specific radioactivity. The recrystallization to constant specific radioactivity also confirmed the efficiency of the chromatographic separation.

Crystallization	5 α -Pregnane-3,20-dione (d.p.m./mg)		Progesterone (d.p.m./mg)		3 β -Hydroxy-5 α -pregnan-20-one (d.p.m./mg)		20 α -Hydroxypregn-4-en-3-one (d.p.m./mg)	
	Crystal	Mother liquor	Crystal	Mother liquor	Crystal	Mother liquor	Crystal	Mother liquor
1st	5710	6120	41780	50420	8330	13290	9280	9990
2nd	5590	5690	42020	41920	8080	8090	9030	9100
3rd	5610	5710	41780	41820	7670	7800	8990	8920
4th	5660	5610	41680	41780	7840	7870	9010	9030

Metabolism of progesterone by rabbit myometrium

Qualitatively the metabolism of progesterone was comparable with that of the endometrium. However, clear quantitative variations were evident (Table 1). Some of the steroids, which were chromatographed in different systems, were further subjected to recrystallization to constant specific radioactivity (Table 2). In the myometrium 7.2% of progesterone was converted per 100mg of tissue whereas 19.5% was converted by the endometrium. The ability of endometrium to metabolize progesterone was thus about 2.7 times that of the myometrium, as calculated from the percentage progesterone converted per 100mg of endometrium and myometrium. As in the endometrial tissue, 3 β -hydroxy-5 α -pregnan-20-one was the major metabolite in the myometrium, constituting about 12.9% of the substrate and about 35.8% of the products formed.

The other major metabolites were 5 α -pregnane-3,20-dione and 20 α -hydroxypregn-4-en-3-one. These constituted about 4.3 and 5.2% of the substrate

respectively, i.e. about 11.9 and 14.4% of products formed. The formation of 5 α -pregnane-3,20-dione was comparatively less in the myometrium than in the endometrium. The reduction of the 3-oxo group, however, appeared to be faster in the myometrium, since 3 β -hydroxy-5 α -pregnan-20-one (35.8% of the products formed) was more than that in the endometrium (26.5%).

Time-study of progesterone metabolism

Fig. 2 shows the metabolism of progesterone in the myometrium after different times of incubation. With the increase in time of incubation, more of progesterone was metabolized. At 5 min incubation only 2–3% of progesterone was converted, whereas 36% was metabolized at 120 min. The first metabolite formed was 5 α -pregnane-3,20-dione, being the major metabolite at the early stages of incubation. Up to 30 min 5 α -pregnane-3,20-dione formation increased and it reached as much as 7.5% of the substrate. It was converted into ring-A saturated hydroxy meta-

bolites, mainly into 3β-hydroxy-5α-pregnan-20-one. At 2h incubation only 4.3% of the total steroid was left as 5α-pregnane-3,20-dione. The formation of 3β-hydroxy-5α-pregnan-20-one was low up to 30 min,

however, at 60 min incubation it was as high as 12.6%. 20α-Hydroxypregn-4-en-3-one was formed gradually in increasing amounts with time. Polar compounds were formed only to a small extent initially and their concentration increased with time.

Fig. 2. Time-study of progesterone metabolism by rabbit myometrium

Myometrial tissue (500 mg) was incubated with [1,2-³H]progesterone (41.8 pmol) for different times at 37°C. The values represent the mean of three observations. The results are expressed as percentage of progesterone converted and the percentage metabolites formed. Progesterone (●); 5α-pregnane-3,20-dione (○); 3β-hydroxy-5α-pregnan-20-one (□), 20α-hydroxypregn-4-en-3-one (■); pregnanediol isomers (▲); origin (Δ).

Metabolism of progesterone in the subcellular preparation of the myometrium

The metabolism of progesterone in different subcellular preparations of rabbit myometrium is shown in Table 3. With the nuclear fraction an average of 16.4% of the progesterone was converted. Progesterone was mainly metabolized to the ring-A saturated 5α-pregnane-3,20-dione. In this fraction it constituted 11.7% of the substrate, which was 71.3% of the products formed. Another metabolite formed in considerable amount was 3β-hydroxy-5α-pregnan-20-one. 20α-Hydroxypregn-4-en-3-one and polar compounds were formed in very low concentrations.

With the mitochondrial fraction 17.7% of progesterone was converted. About 7.4% of progesterone was metabolized to 5α-pregnane-3,20-dione and 3.6% to 3β-hydroxy-5α-pregnan-20-one. 20α-Hydroxypregn-4-en-3-one was only 1.0% and the polar compounds constituted about 5.5% of the substrate.

The metabolism of progesterone with the microsomal fraction was less compared with other subcellular fractions and 14.4% of progesterone was converted. This fraction resembled the mitochondrial fraction in the formation of 5α-pregnane-3,20-dione, 3β-hydroxy-5α-pregnan-20-one and the polar compounds. However, 3β-hydroxy-5α-pregnan-20-one was the major metabolite with this fraction, being about 4% of the substrate.

The conversion of progesterone into its metabolites by the cytosol was greater than with other subcellular fractions. On an average 24% of progesterone was metabolized. In this fraction progesterone was mainly converted into 20α-hydroxypregn-4-en-3-one, which was 16.9% of the substrate, i.e. 70.4% of the products formed.

Table 3. Subcellular metabolism of [1,2-³H]progesterone in the rabbit myometrium

Myometrial tissue (1 g) was fractionated into different subcellular preparations. Each fraction was incubated with [1,2-³H]progesterone (20.9 pmol) in the presence of cofactors. Pregnanediol isomers and highly polar steroids that remained at the origin were designated as 'polar compounds'. The results were expressed as the percentage of progesterone converted and as percentage metabolites formed from the substrate. Values in parentheses are the percentages of the products formed.

Subcellular fractions Steroids	Progesterone converted and metabolites formed (%)			
	Nuclear (800g)	Mitochondrial (10 000g)	Microsomal (105 000g)	Cytosolic (105 000g)
5α-Pregnane-3,20-dione	11.7 (71.3)	7.4 (41.8)	3.4 (23.6)	3.0 (12.5)
Progesterone	16.4	17.7	14.4	24.0
3β-Hydroxy-5α-pregnan-20-one	3.6 (21.9)	3.6 (20.3)	4.0 (27.7)	2.9 (12.1)
20α-Hydroxypregn-4-en-3-one	0.4 (2.4)	1.0 (5.6)	1.3 (9.0)	16.9 (70.4)
Polar compounds	0.3 (1.8)	5.5 (31.1)	3.5 (19.7)	0.8 (3.3)

These studies on the metabolism of progesterone by subcellular preparation clearly showed that the ring-A saturated 5 α -pregnane-3,20-dione is formed in the nuclear fraction, whereas the reduction of the 20-oxo group takes place in the cytosol. The formation of the pregnanolone isomer 3 β -hydroxy-5 α -pregnan-20-one was maximum in the microsomal fraction. The polar compounds accumulated maximally in the mitochondrial and the microsomal fractions. Whether these metabolites are formed in the respective fractions or by contamination of enzymes during homogenization is not very clear.

Effect of oestradiol and progesterone on progesterone metabolism

The metabolism of progesterone in the oestrogen-treated, progesterone-treated and ovariectomized rabbits is shown in Table 4. In the control ovariectomized rabbits the metabolism of progesterone was very slight. Only about 18.5% of progesterone was converted leaving 81.5% as unconverted progesterone. 3 β -Hydroxy-5 α -pregnan-20-one was the major metabolite. With oestradiol treatment, the capacity of the uterus to metabolize progesterone increased significantly. A total of 64.5% of progesterone was metabolized as against 18.5% in the ovariectomized controls. Thus by oestrogen treatment about 3.47 times the amount of progesterone was metabolized than in the ovariectomized controls under standard conditions. 5 α -Pregnane-3,20-dione and 3 β -hydroxy-5 α -pregnan-20-one constituted 19.8% and 13.8% of the substrate, which were 30.7% and 21.4% of the products formed. The metabolism of progesterone to 20 α -hydroxypregn-4-en-3-one was higher in the oestradiol treated animals (3.7%) than in the control (2.2%). However, on a quantitative basis of the percentage of the products formed, the metabolism of progesterone to 20 α -hydroxypregn-4-en-3-one in the oestradiol treated animals was lower (5.7%) than that of the control (11.9%). Accumu-

lation of polar compounds was less in the oestradiol treated than in the control animals.

Oestradiol-primed progesterone-treated animals metabolized 34.5% of progesterone. This was lower than in oestradiol-treated animals (64.5%) and more than in the control rabbits (18.5%). Thus in the progesterone-treated animals the amount of progesterone metabolized *in vitro* was about 1.86 times that in the oestradiol-treated rabbits and 1.86 times that in the control rabbits (calculated from the percentage of progesterone metabolized).

Discussion

The present study has demonstrated unequivocally that progesterone is metabolized in the rabbit endometrium and myometrium. Endometrial tissue metabolized progesterone more than that of the myometrial tissue. Pretreatment of the animals with oestradiol and progesterone revealed that oestradiol *in vivo* stimulated progesterone metabolism *in vitro*, whereas progesterone decreased the metabolism *in vitro*. In the endometrium and myometrium progesterone was metabolized considerably to 5 α -pregnane-3,20-dione. The formation of 5 α -pregnane-3,20-dione from progesterone was demonstrated previously in the rat uterus, pituitary and hypothalamus (Wiest, 1963b, 1969; Armstrong & King, 1971; Karavolas & Herf, 1971; Tabei *et al.*, 1974). The time-study showed that 5 α -pregnane-3,20-dione was the first 5 α -saturated reduction product of progesterone. The reduction of the 4-ene double bond and the formation of 5 α -pregnane-3,20-dione is an enzyme-mediated step. The comparative metabolism of progesterone among the subcellular fractions showed that 5 α -pregnane-3,20-dione was formed in much higher quantity in the nuclear fraction than in other subcellular fractions; this is suggestive of the nuclear localization of the enzyme 5 α -steroid reductase. In the rat hypothalamus (Kara-

Table 4. *Effect of oestradiol and progesterone pretreatment on the metabolism of [1,2-³H]progesterone*

Ovariectomized control rabbits, ovariectomized oestradiol-treated (10 μ g/day for 7 days) rabbits and ovariectomized oestradiol-primed (10 μ g/day for 7 days) progesterone-treated (5 mg/day for 7 days) rabbits were killed and the uterus removed. The myometrial tissue (500 mg) was minced and incubated with [1,2-³H]progesterone (41.8 pmol) for 2 h at 37°C. The results are expressed as percentage of progesterone converted and as percentage metabolites formed from the substrate. Values in parentheses are the percentages of the products formed.

Steroids	Ovariectomized control	Oestradiol treated	Progesterone treated
5 α -Pregnane-3,20-dione	1.0 (10.3)	19.8 (30.7)	5.7 (16.5)
Progesterone	18.5 3.7*	64.5 12.9*	34.5 6.9*
3 β -Hydroxy-5 α -pregnan-20-one	4.0 (21.6)	13.8 (21.4)	9.1 (26.4)
20 α -Hydroxypregn-4-en-3-one	2.2 (11.9)	3.7 (5.7)	2.5 (7.2)
5 α -Pregnane-3 β ,20 α -diol	1.7 (9.2)	12.0 (18.6)	6.2 (17.9)
Origin	6.3 (34.1)	14.0 (21.7)	7.3 (21.2)

* Percentage progesterone converted per 100 mg of tissue.

volas & Herf, 1971) and in the rabbit hypothalamus (Laumas *et al.*, 1974) similar conversion of progesterone into 5 α -pregnane-3,20-dione has been reported in the nuclear fraction. The smaller activity observed in the mitochondrial and microsomal fractions may be due to contamination of the nuclear membrane during homogenization.

The maximal formation of 3 β -hydroxy-5 α -pregnan-20-one after 30 min of incubation suggested that after the initial conversion of progesterone into 5 α -pregnane-3,20-dione, the oxygen function at C-3 of the molecule was subsequently reduced by the enzyme 3 β -hydroxy steroid dehydrogenase. Thus the rabbit uterus differs from that of the rat uterus where 3 α -hydroxy-5 α -pregnan-20-one was formed (Wichmann, 1967; Armstrong, 1973) and the human endometrium and myometrium where 20 α -hydroxy-5 α -pregnan-3-one was formed as the reduction product of 5 α -pregnane-3,20-dione (Bryson & Sweat, 1967; 1969; Collins *et al.*, 1969; Collins & Jewkes, 1974; Verma & Laumas, 1975), whereas in the pig uterus, as in the rabbit uterus, the presence of the enzyme 3 β -hydroxy steroid dehydrogenase has been reported (Henricks & Tindall, 1971). The formation of 3 β -hydroxy-5 α -pregnan-20-one in large quantities in the microsomal and mitochondrial fractions suggested that the enzyme 3 β -hydroxy steroid dehydrogenase is localized in these fractions. The formation of 3 β -hydroxy-5 α -pregnan-20-one and the presence of the enzyme 3 β -hydroxy steroid dehydrogenase has been demonstrated in rat liver and adrenal glands (Rubin *et al.*, 1961; Jackanicz & Armstrong, 1968) and in rabbit ovarian microsomal preparations and mitochondria (Basch & Finegold, 1971).

In the rabbit uterus 20 α -hydroxypregn-4-en-3-one was another major metabolite of progesterone. The conversion of progesterone into 20 α -hydroxypregn-4-en-3-one *in vivo* in the rabbit tissues was shown previously (Cooke *et al.*, 1963; Allen *et al.*, 1967; Macartney & Thomas, 1969; Verma, 1973). The conversion of progesterone into 20 α -hydroxypregn-4-en-3-one in the cytosol suggested the presence of the enzyme 20 α -hydroxy steroid dehydrogenase in this fraction. In the rat uterus, ovary, liver and pituitary (Wiest, 1963b; Wichmann, 1967; Tabei *et al.*, 1974) this enzyme has been reported in the cytosol fraction. The time-study suggested that the conversion of progesterone into 20 α -hydroxypregn-4-en-3-one is another pathway of progesterone metabolism, simultaneously operating in the cytosol.

The treatment of rabbits with oestradiol and progesterone demonstrated that oestradiol priming enhances the progesterone metabolism and pre-treatment with progesterone decreases the progesterone metabolism. A decrease in the conversion of progesterone during dioestrus and pseudopregnancy has been shown in the rat uterus (Wiest, 1969). An increase in the metabolism of progesterone as an effect of

oestradiol has been reported by Clark (1974) in the mouse uterus and by Armstrong (1973) in the rat uterus. These actions of oestradiol and progesterone on the metabolism of progesterone may be brought about by their effect on progesterone receptor synthesis. Oestradiol facilitates progesterone receptor synthesis (King & Mainwaring, 1974; Rao *et al.*, 1974; Reel & Shih, 1974), whereas it has been suggested that progesterone inactivates the receptor (Milgrom *et al.*, 1972; Young & Cleary, 1974). Thus oestradiol may enhance the progesterone metabolism by elevating the enzymes for progesterone metabolism, whereas progesterone does not maintain the levels of enzymic systems induced by oestrogen.

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