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PROGESTERONE METABOLISM IN THE HUMAN MYOMETRIUM*

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SUMMARY

1. The metabolism of [4-C^{14}]-progesterone in minced uterus, brei and homogenate preparation of human myometrium has been investigated with and without addition of cofactors using a steroid–tissue ratio between 1 : 1000 and 1 : 834,000.

2. After purification of the extracts on Amberlite XAD-2 or by solvent partition, the metabolites or groups of metabolites were separated by thin-layer chromatography or gel column chromatography on Sephadex LH-20.

3. The metabolites were identified by their behaviour on thin-layer, gel column and gas chromatography, and by radio gas chromatography or mass spectrometry.

4. Progesterone was metabolized under all the experimental conditions employed in this work; the following metabolites have been characterized or identified: 5α-pregnane-3,20-dione, 20ξ-hydroxy-5α-pregnan-3-one, 20α-hydroxy-4-pregnen-3-one, 6β-hydroxy-progesterone, 5α-pregnane-3β,20α-diol and 5α-pregnane-3α,20α-diol. The possible formation of other unidentified metabolites is discussed.

INTRODUCTION

The ability of the human uterus to metabolize progesterone has been accepted since the experiments by Bryson and Sweat with endometrial [1, 2] and myometrial [3] tissue collected during the proliferative phase of the ovarian cycle.

In contrast to these results Jonat *et al.*[4] and Egert and Maass[5] were unable to detect any progesterone metabolite after *in vitro* incubation of this steroid, either with endometrial or myometrial human tissue or with rat uterine tissue [4]. Even after injection of [^{3}H]-progesterone Egert and Maass[5] found no progesterone metabolites in the uterine tissue of eviscerated pregnant and non-pregnant rats.

The demonstration of specific progesterone binding proteins in the rat [6–9], guinea-pig [6], mouse [9], rabbit [7, 8, 10], and calf [11] uterus as well as human endometrium [10], and of the competition of 5α-pregnane-3,20-dione with progesterone for binding sites on its receptor in the human endometrium cytosol [10], raises the question of the metabolism of progesterone in the human uterus, and emphasizes the need for re-investigation of this problem.

MATERIAL AND METHODS

Steroids

Non labelled reference steroids were obtained from the following sources: Progesterone (4-pregnene-3,10-dione), 20β-dihydroprogesterone (20β-hydroxy-4-pregnen-3-one), 17-hydroxyprogesterone (17-hydroxy-4-pregnene-3,20-dione), pregnanedione, (5β-pregnane-3,20-dione), allopregnanedione (5α-pregnane-3,20-dione) and allopregnanediol (5α-pregnane-3α,20α-diol) were from Merck AG, Darmstadt, Germany; 20α-dihydroprogesterone (20α-hydroxy-4-pregnen-3-one) and pregnanediol (5β-pregnane-3α,20α-diol) were from Schering AG, Berlin, Germany; 6β-hydroxyprogesterone (6β-hydroxy-4-pregnene-3,20-dione), 5α-pregnane-3β,20α-diol, 5β-pregnane-3α,20α-diol, 5β-pregnane-3β,20β-diol and 20β-hydroxy-5α-pregnan-3-one were from Steraloids Inc., Flushing, N.Y., U.S.A. 5α-pregnane-3α,6β,20α-triol was a gift from Dr. Thomas, England.

Labelled [4-C^{14}]-progesterone was purchased from New England Nuclear Chemicals GmbH, Dreieichenhain, Germany (article no. NEC-081) and had a specific activity of 52,8 mCi/mmol. The purity of this compound was assayed by thin-layer and radio gas chromatography.

Non steroids

All the solvents and reagents used in this investigation were of highest purity (analytical grade) and were purchased from Merck AG, Darmstadt, if not otherwise stated. Pyridine was redistilled before use. dl-Isocitrate trisodium salt and chlorotrimethylsilane were from Schuchardt-Merck, München, Germany; isocitrate dehydrogenase (10% solution in glycerol), NADP and NADH were from Boehringer, Mannheim, Germany; methoxyamine hydrochloride and 1,1,1,3,3,3-hexamethyldisilazane were from Eastman, Kodak, Co., U.S.A. Amberlite XAD-2 (300–1000 μm) was from Serva, Heidelberg, Germany, and was successively washed with methanol, acetone and water before use.

Chromatographic techniques

(a) *Thin-layer chromatography.* Single or multiple one dimensional t.l.c. was carried out on 20 × 20 cm

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precoated silica gel layers (silica gel 60, Merck, Darmstadt, 0·25 mm thick) according to the specifications of Lisboa[12, 13]; for the investigation of the incubations the following solvent systems [14] were used: chloroform–ethanol 98 : 2 v/v (single migration) and benzene–ethanol 98 : 2 v/v (multiple migrations). Authentic standards running simultaneously with the extracts were detected under U.V.-light (254 nm) or after development with the anisaldehyde–sulfuric acid–acetic acid reagent [12, 13]. Radiochromatograms were scanned by a thin-layer-scanner with methane flow (Labor. Prof. Berthold, Wildbad, Germany) model IILB 2722, with counting tube LB 2007/LB6280 under the following experimental conditions: methane flow: 12 ml/min; time constant 10 sec, measure range variable between 1 and 30 c.p.s., paper speed 300 mm/h, split 1 mm. 4-ene-3-oxo-steroids were scanned by reflectance spectrometry at 240 nm on a Zeiss-chromatogram spectrophotometer (Carl Zeiss, Oberkochen, Germany).

(b) *Gel column chromatography.* Gel column chromatography on 4 or 8 g Sephadex LH-20 (particle size 25–100 μm, Pharmacia, Fine Chemicals, Sweden) was carried out on 1 × 30 cm glass columns [15] using solvent systems: *S-1* cyclohexane–benzene–methanol 80:15:5 by vol., *S-2* n-hexane–ethyl-acetate–methanol 90:7·5:2·5 by vol. and *S-3* n-heptane–chloroform–ethanol 50:50 by vol.; flow rate: 0·5–0·6 ml/min (S-1, S-2) or 0·4 ml/min (S-3); fractions of 2·5 ml were collected in all chromatograms. Table 1 shows the elution pattern of some steroids in these systems under the experimental conditions used in this work (Table 1).

For identification purposes the specific activity of the radiometabolites was measured in the eluates obtained from the Sephadex columns, in which cold standards and isolated labelled metabolites were chromatographed simultaneously. Aliquots of each eluate were assayed for radioactivity in 10 ml of Instagel (Packard Co., U.S.A.) in a scintillation counting spectrometer (model 3380 Packard Co., U.S.A.): cold steroids were measured by U.V.-absorption at 240 nm (4-ene-3-oxo-steroids). Pregnanediol was measured at 425 nm by a colorimetric procedure according to Klopper [16] which develops sulfuric acid chromogenicity.

(c) *Gas–liquid chromatography.* Gas chromatography was carried out on a Pye gas chromatograph (Pye Unicam Ltd, England) after trimethylsilylether (TMSi-ether) and methoxime–trimethylsilylether (Mo TMSi-ether) derivative formation. For specifications see a previous paper [17].

(d) *Gas chromatography–mass spectrometry.* Gas chromatographic analysis was combined with mass spectrometry in a LKB 2091 gas chromatograph–mass spectrometer interfaced with a PDP-11 computer (LKB-Produkter AB, Sweden). After formation of TMSi–ether derivatives, the analysis was performed at 170°C on 270 × 0·4 cm columns packed with gaschrom Q and coated with 1% OV-17 phase with helium as carrier gas; the temperature of the ion source was 200°C, the ionization energy 70 eV and the ionization current 50 μA.

(e) *Radio gas chromatography.* Radio gas chromatography was carried out on a Pye-gas chromatograph series 104 associated with the detector assembly for radio gas chromatography manufactured by Panax Equipment Ltd, England. The splitter between FID and radio section was 1:10 and the temperature of the flowing reactor was 650°C. The furnace tube was filled with cupric oxide. Carbon dioxide (2 ml/min) was used as quench gas and argon as carrier (48 ml/min).

Myometrium preparations

Macroscopically normal myometrium tissue obtained immediately after hysterectomy was separated from endometrium, washed in physiological sodium chloride solution and minced in small portions. Myometrium brei was prepared by homogenizing 4 10 g minced tissue at 4°C in an Ultra-Turrax apparatus with a Krebs–Ringer-glucose solution (containing 20 mmol/l glucose per litre to a final vol. of 30 ml. Under similar conditions a further myometrium brei with 0·25 mol/l sucrose was prepared; this brei was used for preparations of a 10% homogenate suspension using a Potter–Elvehjem homogenizer with Teflon pestle at a speed of 1·500 rev./min.

Table 1. Elution volumes of several C_{21}-steroids on Sephadex LH-20 using the solvent systems S-1, S-2 and S-3 under the experimental conditions described in the text. Elution fractions of 2·5 ml were collected and analysed by thin-layer chromatography on cyclohexane–ethyl-acetate–ethanol 45 : 45 : 10 by vol. or chloroform–ethanol 92 : 8 v/v

Steroids	Solvent systems S-1/4 g	S-2/4 g	S-2/8 g	S-3/4 g
Allopregnanedione	5–12·5		15–30	
Progesterone	5–20	10 27·5		15–20
20β-Hydroxy-5α-pregnan-3-one		12·5–27·5	20 55	17·5–27·5
3β-Hydroxy-5α-pregnan-20-one			20 70	
20α-Dihydro-progesterone	20–35	30–57·5	60 95	22·5–35
20β-Dihydro-progesterone		20–42·5	45–75	22·5–35
6β-Hydroxyprogesterone	35–50	60 97·5	80–170	42·5–55
17α-Hydroxyprogesterone		55 90		
5α-Pregnane-3α,20α-diol			65–105	
5α-Pregnane-3β,20α-diol			75–165	
5β-Pregnane-3α,20α-diol	35 45	75–97·5		55–77·5

Conditions of incubations

The preparations of human myometrium with 1 μCi of [4-^{14}C]-progesterone and/or nonlabelled progesterone were incubated as follows:

I. Incubation with minced myometrium. One microcurie of progesterone was incubated without addition of cofactors at 37°C for 30 min with minced myometrium corresponding to 5 g tissue in 30 ml of a Krebs–Ringer-glucose solution (steroid–tissue ratio 1:834,000).

II. Incubation with myometrium brei. A preparation containing 5 g tissue/15 ml Krebs–Ringer-glucose solution (pH 6·8) was incubated at 37°C for 60 min with (IIa) 5 mg progesterone (dissolved in propyleneglycol) or (IIb) 1 μCi labelled and 5 mg unlabelled progesterone after addition of 1 ml 0·1 mmol/l $MnCl_2$, 1 ml 0·2 mol/l NADP, 1 ml 0·05 mol/l sodium isocitrate and 1 ml 0·2% isocitrate-dehydrogenase (in glycerol). The incubations IIa and IIb had a steroid–tissue ratio of 1:1000. Furthermore (IIc), a brei preparation containing 2 g tissue/15 ml Krebs–Ringer glucose solution (pH 6·8) with the addition of 1 ml 0·1 mmol $MnCl_2$ was incubated with 1 μCi progesterone (dissolved in 1 ml Krebs–Ringer glucose solution) without addition of cofactors at 37°C for 60 min (steroid–tissue ratio 1:333,600).

III. Incubation with homogenate preparation. 20 ml of this preparation was incubated with 1 μCi and 2 mg progesterone at 37°C for 90 min in an incubation medium containing 5 ml 0·5 mol/l Tris–HCl buffer (pH 7·5), 2 ml 0·1 mol/l $MgCl_2$, 2 ml 0·1 mmol/l $MnCl_2$, 2 ml 1 mol/l nicotinamide, 2 ml 0·02 mol NADH, 2 ml 0·02 mol/l NADP, 4 ml 0·05 mol/l sodium isocitrate and 0·4 ml of a 10% isocitrate dehydrogenase solution (in glycerol) (steroid–tissue ratio 1:1000).

Incubation set I and II were stopped by adding 10 ml of ether–chloroform (3:1 v/v); incubation III was stopped by adding methanol to a final volume, incubation medium: methanol of 9:1. All the incubations were cooled at −5°C until the beginning of extraction and purification.

Purification of extracts. The incubations I and II (a, b, c) were extracted with ether–chloroform 3:1 and the sediment obtained from the incubation phase after centrifugation was further extracted with chloroform and methanol. The pooled organic phases were further purified according to the method of Fukayama *et al.*[17]. The details can be taken from a previous paper [18].

The radioactivity was distributed between the petroleum ether phase (10 to 17%) and the ethyl acetate phase (82 to 89%); less than 1% of total radioactivity was recovered in the water phase. Since it could be shown that progesterone and less polar materials (as for instance steroids isopolar to 5α or 5β-pregnanedione), which were present in the petroleum ether phase, would also be present in the ethyl acetate phase, only the ethyl acetate extract was employed for identification purposes in this investigation.

The incubation III was purified on 50 g Amberlite XAD-2 (100–200 μm) columns according to the method first described by Bradlow *et al.*[19]. After addition of methanol to the incubations up to a final volume water–methanol 9:1 (4 ml were added to the incubation medium) the mixture was centrifuged and the precipitate washed two times with 5 ml water–methanol 9:1. The pooled supernatants were added to a column (1 × 15 cm) packed with 50 g Amberlite in water, giving a flow rate of 0·5–0·7 ml/min. The Amberlite column was washed with 25 ml water, 25 ml water–methanol 9:1 and finally with 75 ml pure methanol. This fraction contained the purified steroids.

RESULTS

(a) *Incubations with minced myometrium* (I) *and brei* (IIa, b, c) *preparations*

The ethyl-acetate extracts obtained from the incubations with minced (I) and brei (IIa) preparations were subjected to column gel chromatography on Sephadex LH-20 in solvent system S-1. Besides huge amounts of unmetabolized progesterone at least three groups of metabolites more polar than progesterone were demonstrated in these chromatograms: fraction A, elution volume between 21 and 39 ml, isopolar to 20α-(and β-)hydroxy-4-pregnen-3-ones, fraction B, 40–56 ml, isopolar to 6β-hydroxyprogesterone and 5α-pregnane-3α,20α-diol, and fraction 3, 57–72 ml. A column radio chromatogram of the purified ethyl acetate extract from incubation I is reproduced in Fig. 1a. The distribution of radioactivity among the metabolites more polar than progesterone is very similar in the ethyl acetate and petroleum ether phases as can be seen in the radio chromatogram of the incubation IIa (Fig. 1 b). This purification procedure was used during the isolation and identification of the polar metabolites. The ethyl-acetate extract from incubation IIa, which was carried out with nonlabelled progesterone, was chromatographed on Sephadex LH-20 in solvent system S-1, and several fractions were examined by gas chromatography. The fractions corresponding to 6–9, 10–12 and 13–15 ml eluates showed chromatographic peaks with rrt-values (5α-cholestane: 100) of 5α-pregnane-3,20-dione-bis-methoxime on both, SE-30 (1·03) and QF-1 (1·39), besides considerable amounts of the incubated steroid (SE-30, 114; QF-1, 1·70). The fraction collected between 42–49 ml gave evidence for the presence of 6β-hydroxyprogesterone as both, TMSi (rrt-values on QF-1: isolated steroid 8·5; standard 8·7; SE-30: 1·03) and MoTMSi-ether (QF-1 1·46; SE-30 1·08–1·10). Typical gas chromatograms of the Mo-TMSi-ether derivative of the compound identified as 6β-hydroxyprogesterone are shown in Fig. 2 (a, b). Mass spectrometric analysis of this sample after silyl-ether derivative formation showed a mass spectrum typical of 6-hydroxyprogesterone presenting a molecular ion at m/e 402 (abundance 30%), a base peak at m/e 387 (M–15) and an ion peak at m/e 346 (abundance 74%) which corresponds to M-56.

The metabolites found in the fractions A and B in the incubations carried out with minced (I) and brei

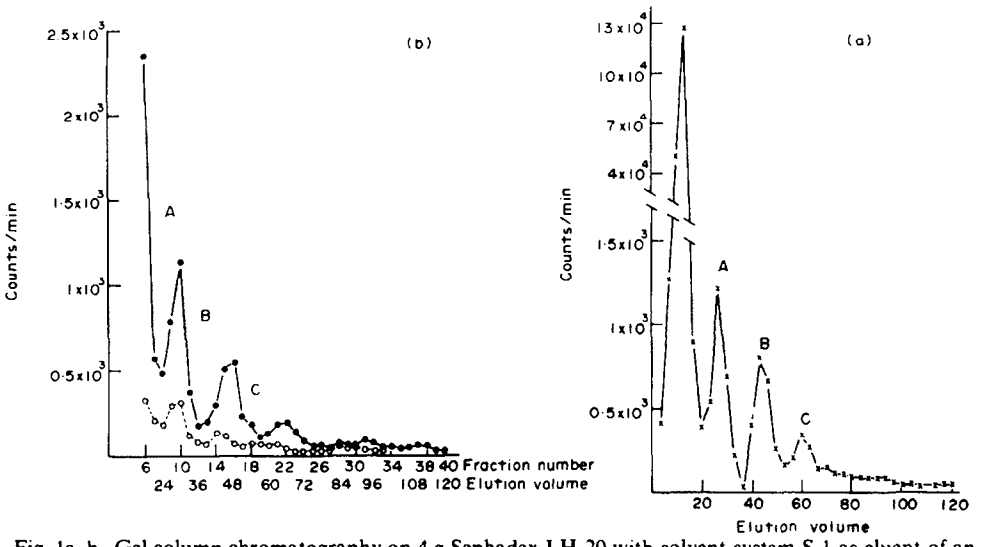

Fig. 1a, b. Gel column chromatography on 4 g Sephadex LH-20 with solvent system S-1 as eluent of an aliquot of the ethyl-acetate extract obtained after incubation of 1 μCi [4-C^{14}]-progesterone (and 5 mg nonlabelled steroid) with 5 g minced human myometrium tissue (1a) or of aliquots of both, ethylacetate (-●-●-) and petroleum ether (-○-○-) extracts obtained after the incubation of 1 μC [^{4}-C^{14}]-progesterone with a brei preparation of human myometrium corresponding to 5 g tissue (1b). Radioactivity was determined in 1/10 aliquots of each 2·5 ml fraction by liquid scintillation counting. Eluate fractions 21–39 ml (A), 40–56 ml (B) and 57–72 ml (C) are isopolar to 20-hydroxy-4-pregnen-3-ones (A), 6β-hydroxyprogesterone/5α-pregnane-3β(or 3α)20α-diol (B) and a dihydroxy-monoketonic steroid (C). The fractions 2–5 (2·5 to 10·5 ml) corresponding to incubated progesterone (and less polar metabolites) are not reproduced in Fig. 1b.

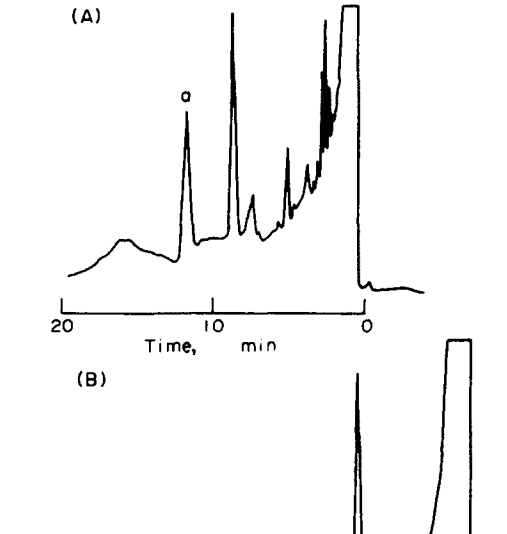

Fig. 2. Gas–liquid chromatography (g.l.c.) of the fraction 42–49 ml obtained from gel column chromatography on Sephadex LH-20 developed with solvent system S-1, of the ethylacetate extract obtained after incubation of non-labelled progesterone with a brei preparation of human myometrium (steroid–tissue ratio 1:1000). The gas chromatogram was obtained after methoxime–silyl–ether derivative formation on a 3% QF-1 (A), or on a 2·2% SE-30 (B) phase. The peaks corresponding to 6β-hydroxyprogesterone derivatives are indicated by (a). The double peak in the B chromatogram designated *a* corresponds to the anti- and syn-geometric isomers of 6β-hydroxyprogesterone Mo–TMSi–ether.

(IIc) preparations were further analysed by rechromatography on Sephadex LH-20. In both incubations the radioactive material of fraction A showed two metabolites with the polarities of 20-hydroxy-5α-pregnan-3-one (12·5–20 ml) and 20α-hydroxy-4-pregnen-3-one (20–50 ml) after gel chromatography on 4 g Sephadex LH-20 in system S-2. Figure 3 shows the column radio chromatogram obtained from a mixture of an aliquot fraction A (peak A of the brei incubation) and 200 μg of 20α-hydroxy-4-pregnen-3-one. The specific activity in the fractions 11 to 18 (27·5–45 ml) was the same when the radioactivity of the isolated material determined by liquid scintillation counting was compared with the amount of added steroid measured by its absorption at 240 nm.

Fraction B of the incubations I and IIb was separately submitted to gel column chromatography on 8 g Sephadex LH-20 in the solvent system S-3. There were four groups of metabolites with the following elution volumes: (a) 15–35 ml, isopolar to 20α-hydroxy-4-pregnen-3-one, a steroid which had been isolated in the previous fraction A; (b) 40–55 ml, isopolar to 6β-hydroxyprogesterone and 5α-pregnane-3α,20α-diol; (c) 60–75 ml, with a maximum of radioactivity in fraction 27 (67·5 ml), corresponding with a relative retention volume of 1·35 (rrv) to 6β-hydroxyprogesterone isopolar to 5α-pregnane-3β,20α- and 5β-pregnane-3α,20α-diol, and (d) 75–85 ml, with a polarity similar to dihydroxymonoketonic C_{21}-steroids, as for instance 6β,20β-dihydroxy-4-pregnen-3-one.

The steroids found in the zones b and c were identified in the fraction B of the incubation IIa, which was chromatographed under the same conditions as described above, but with the addition of 200 μg of 6β-hydroxy-

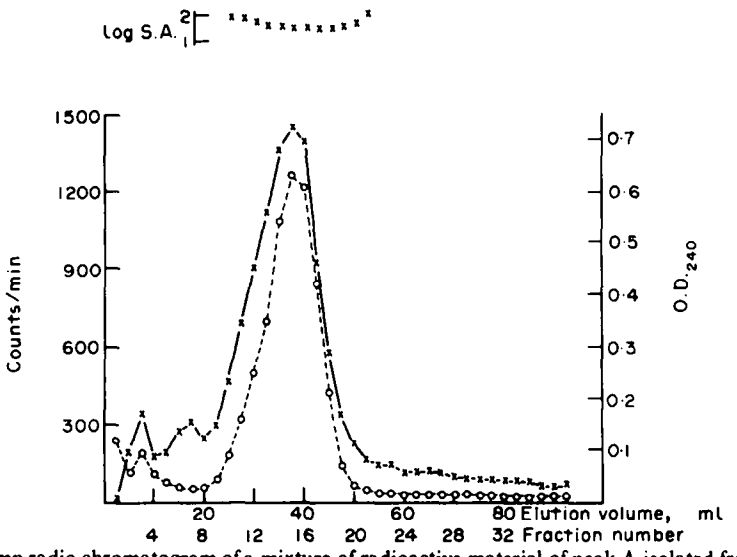

Fig. 3. Column radio chromatogram of a mixture of radioactive material of peak A isolated from incubations of progesterone with brei preparations of human myometrium and 200 μg authentic 20α-hydroxy-4-pregnen-3-one, obtained on 4 g of Sephadex LH-20 in solvent system S-2. Comparison of the radioactivity found in the fractions 11–18 and the amount of added steroid which was measured by its absorption at 240 nm showed a constancy of the specific activity in all these fractions (27·5–45 ml). A small peak observed for the fractions 5–8 is isopolar with 3α-hydroxy-5α-pregnan-20-one.

progesterone and 200 μg of 5β-pregnane-3α,20α-diol (Fig. 4). The specific radioactivity (c.p.m./weight of steroid) calculated by comparing the radioactivity measured by liquid scintillation counting with the U.V.-absorption at 240 nm of the added standard in all 2·5 ml fractions eluted between 42·5 and 55 ml (fractions 17 to 22) indicated the presence of 6β-hydroxyprogesterone in zone b; however, the presence of small amounts of 5α-pregnane-3α,20α-diol in this peak could not be excluded because the rrv of 5α-pregnane-3α,20α-diol to 6β-hydroxyprogesterone in this system is 1·00. The steroids present in the zone c were slightly less polar than the added 5β-pregnane-3α,20α-diol, which was added as internal standard and measured in the eluates after development of sulphuric acid chromogenicity. Comparison of the rrv of 5α-pregnane-3β,20α-diol (1·35) and 5β-pregnane-3α,20α-diol (1·41) to 6β-hydroxyprogesterone and that of the isolated metabolite (1·35) indicate an identity of the isolated pregnanediol with 5α-pregnane-3β,20α-diol.

The ethyl acetate extract of the incubation IIb (5 mg progesterone, 1 μCi, with a brei preparation of myometrium corresponding to 5 g tissue; steroid–tissue ratio 1:1000) was investigated by thin-layer chromatography (system chloroform–ethanol 97:3; silica gel G). Besides unmetabolized progesterone, two principal

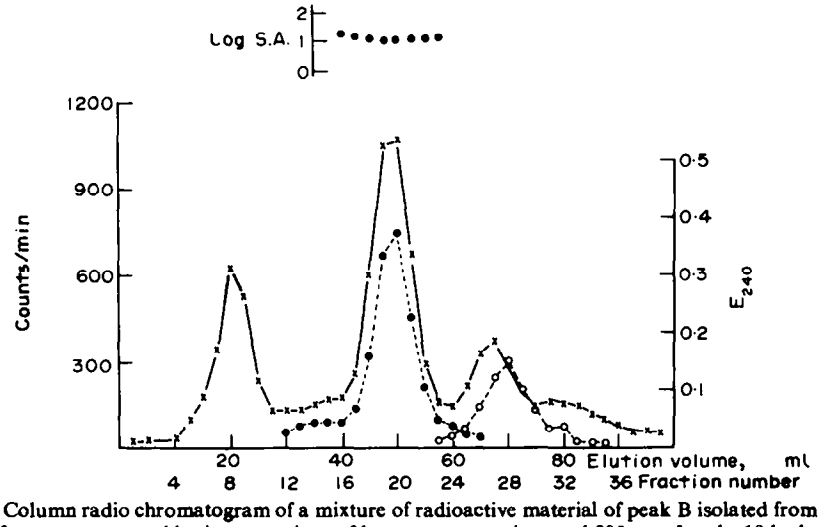

Fig. 4. Column radio chromatogram of a mixture of radioactive material of peak B isolated from incubations of progesterone and brei preparations of human myometrium and 200 μg of each, 6β-hydroxy-progesterone and 5β-pregnane-3α,20α-diol, obtained on 8 g Sephadex LH-20 in solvent system S-3. Comparison of the radioactivity of fractions 17 to 22 and the amount of added 6β-hydroxyprogesterone which was measured by its absorption at 240 nm shows a constancy of the specific activity in all these fractions (42·5–55 ml). A discrepancy was found between the elution volume of authentic 5β-pregnane-3α,20α-diol and the radiometabolite eluted between 60 and 70 ml, the polarity of which is quite identical to that of 5α-pregnane-3β,20α-diol.

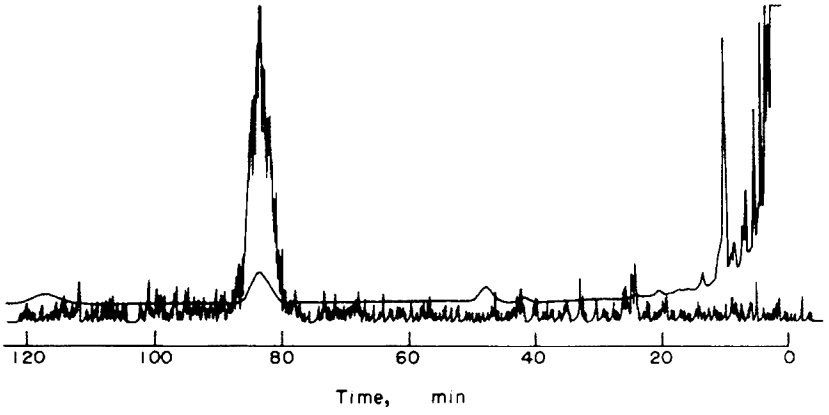

Fig. 5. Radio gas chromatography of a fraction isopolar to 6β-hydroxyprogesterone isolated after t.l.c. on silica gel G (chloroform–ethanol 97:3) of ethylacetate extracts from the incubation of myometrium brei and progesterone. Isolated material and authentic 6β-hydroxyprogesterone were chromatographed simultaneously after TMSi-ether formation at 205°C on a 3% QF-1 column. For details see under Material and Methods.

radio peaks were found with mobility values of 3·5 cm (hR_F = 24) and 4·8 cm (hR_F = 32), both U.V.-positive at 240 nm. The less polar peak was isopolar to 20α-hydroxy-4-pregnen-3-one and the more polar one to 6β-hydroxyprogesterone. The presence of both steroids was confirmed by radio gas chromatography. The fraction which in the t.l.c. had a mobility value between 3·3 and 4·1 cm corresponding to the radio peak with a hR_F value of 24, was gas-chromatographed on a 3% QF-1 phase at 205°C together with authentic 6β-hydroxyprogesterone after trimethyl silyl ether formation. The isolated radio metabolite and 6β-hydroxyprogesterone were completely identical, as can be seen in the radio gas chromatogram reproduced in Fig. 5.

Another t.l.c.-fraction contained a metabolite (mobility value between 2·6 and 3·3 cm from the starting line) slightly more polar than the previous one; it was isopolar to 5α-pregnane-3α,20α-diol on QF-1 (rrt to cholestane: as TMSi-ether 0·79, absolute retention value for cholestane, 7 min, 215°C). For comparison, some rrt-values of other epimeric pregnanediol TMSi-ether, measured under the same experimental conditions, were: 5α-pregnane-3β,20α-diol, 1·13; 5α-pregnane-3β,20β-diol, 1·04; 5β-pregnane-3α,20α-diol, 0·95; 5β-pregnane-3β,20β-diol, 0·76.

(b) *Incubation with homogenate preparation*

Aliquots of the incubation carried out with homogenate preparation were investigated by ascending t.l.c. on silica gel G developed in chloroform–ethanol 98:2. As shown in Fig. 6, besides a number of minor peaks at 1·5, 3·5 and 6·5 cm from the starting line, four principal peaks were found to be isopolar with 6β-hydroxyprogesterone, 20α-hydroxy-4-pregnen-3-one, 5α-pregnane-3,20-dione and progesterone, respectively. Peaks for the radioactive metabolites 4-ene-3-oxo-groups were also found after scanning of the chromatoplate by reflectance spectrometry at 240 nm. Proof of identity of these steroids was obtained by radio gas chromatography in a similar manner as described above for the incubations with brei preparations. A new proof for the identity of 5α-pregnanedione in the less polar fraction of this chromatogram (8·5–10 cm.) was obtained by repeated chromatography on a silica gel layer developed in benzene–ethanol 98:2, as can be seen in Fig. 7.

To evaluate the yield of metabolization of progesterone (steroid–tissue ratio 1:1000), an aliquot of this incubation was subjected to gel column chromatography on 8 g Sephadex LH-20 using 350 ml of solvent system S-2. Ninety-four per cent of the radioactivity was found in the elution fractions 7–20 (15–50 ml) corresponding to unmetabolized progesterone and less polar steroids. 3·27 per cent in the fraction isopolar to 20α (and 20β)-hydroxy-4-pregnen-3-one, 3β-hydroxy-5α-pregnan-20-one and 5α-pregnane-3α,20α-diol (51–

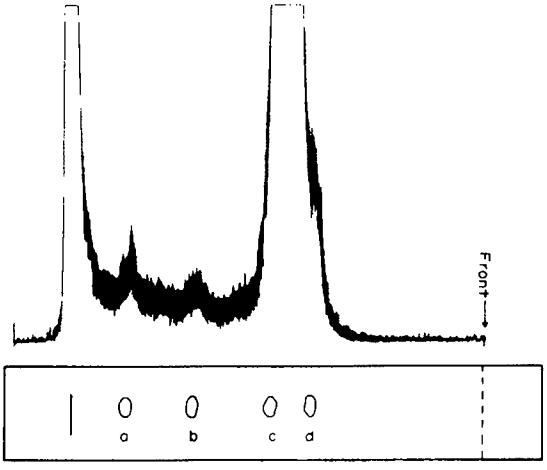

Fig. 6. Ascending thin-layer chromatogram of an aliquot of the purified extracts obtained from the incubation of 1 mCi and 5 mg [^{14}C]-progesterone with a homogenate preparation of human myometrium. The silica gel chromatogram was irrigated with chloroform-ethanol 98:2 and radioscanned with methane flow (measure range 30 c.p.s., time constant 10 sec; 12 mm/h). In the lower part the chromatography of standards 6β-hydroxyprogesterone (a), 20α-hydroxy-4-pregnen-3-one (b), progesterone (c) and 5α-pregnane-3,20-dione (d).

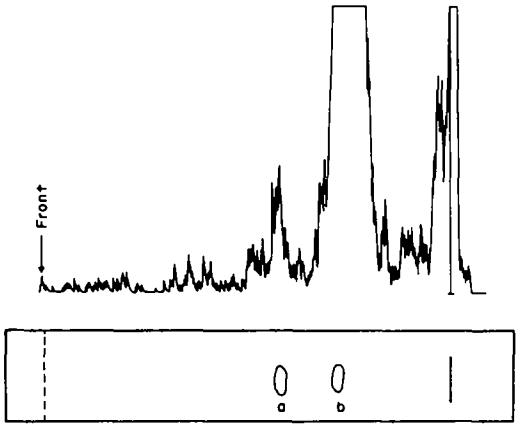

Fig. 7. Radioscanning of a multiple ascending t.l.c. (run twice in benzene–ethanol 98:2) of the less polar fraction (8·5–10 cm) which was scraped off a previous chromatogram in which the incubation of [^{14}C]-progesterone and a homogenate preparation of human myometrium was investigated. The less polar peak in this chromatogram, presenting a hRs-value to progesterone of 152, is identical to 5α-pregnane-3,10-dione.

100 ml), and 1·25% in the fraction isopolar to 6β-hydroxyprogesterone and 5α-pregnane-3β,20α-diol (101–187,5 ml). Less than 1·5% were found in the fractions 188–350 ml corresponding to steroids with a dihydroxy-monoketonic of trihydroxy groups.

DISCUSSION

The results reported demonstrate that 5α-ring-reductase, 3α,3β-and 20α-hydroxysteroid-oxidoreductase as well as 6β-hydroxylase, all acting on progesterone as substrate, are present in the human myometrium of the early post-menopause and thereby confirm and extend by new analytical methods the results of other investigators [1–3].

Bryson and Sweat[3] have isolated and identified, by crystallization data, 5α-pregnane-3,20-dione, 20α-hydroxy-4-pregnen-3-one and 6β-hydroxyprogesterone as metabolites of progesterone incubated with myometrium obtained during the proliferative phase of the ovarian cycle; in the same experiments they have presented paper chromatographic evidence for the formation of 20α-hydroxy-5α-pregnan-3-one and one more polar unidentified metabolite.

In the present investigation we have isolated and characterized, by column gel, thin-layer and/or gas–liquid chromatography, 5α-pregnane-3,20-dione, 20α-hydroxy-4-pregnen-3-one, 3α-hydroxy-5α-pregnan-20-one, 6β-hydroxyprogesterone and 3β-allopregnanediol in incubations of progesterone with myometrium preparations collected in the early post-menopause. Two of these steroids—20α-hydroxy-4-pregnen-3-one and 6β-hydroxyprogesterone—were identified by calculation of specific activity in the eluent fractions obtained from the column gel chromatography on Sephadex LH-20 after simultaneous chromatography of radioactive material and authentic carrier, and by radio gas chromatography. In addition, gas chromatographic-mass spectrometric data support the identification of the $C_{21}O_3$-metabolite as 6β-hydroxyprogesterone. 20β-hydroxy-4-pregnen-3-one, a progesterone metabolite found by Sweat *et al.*[20] in cultured human uterine fibroblast, and by Sweat and Bryson[21] in incubations with human endometrium of proliferative phase, has not been detected in our incubations.

In pooled extracts of human myometrium of early pregnancy Runnebaum and Zander[22] have isolated, besides progesterone (111 ng/g wet tissue), minute amounts of 20α-hydroxy- and 20β-hydroxy-4-pregnen-3-one (5 and 0·28 ng/g respectively). The failure to isolate 20β-dihydroprogesterone in this investigation does not imply the absence of this metabolite in our preparations, but perhaps its production in very small amounts.

In spite of progestational activities demonstrated for both 20α- and 20β-dihydroprogesterone in bioassay tests [23], Wiest and collaborators have found a lack of progestational effect of the former in pregnant rats [24] as well as of both epimers in ovariectomized mice [25]. However, as an inactive steroid, 20α-dihydroprogesterone could be a factor regulating the amount of progesterone present in the target tissue, as was shown for the human foetoplacental unit [26, 27].

The isolation of 5α-pregnane-3,10-dione as a metabolite of progesterone in its target tissue has some similarity with observations on testosterone in the prostate. In castrated pseudopregnant rats Sanyal and Villee[28] have observed that 5α-pregnane-3,20-dione—but not 3β-hydroxy-5α-pregnan-3-one or 5α-pregnane-3β,20α-diol—has a slight stimulatory effect on uterine weight, approximately one third of that observed for progesterone. The progestational properties of progesterone disappear, however, with the 5α-reduction of ring A; therefore 5α-dihydroprogesterone does not stimulate the development of decidual cells in pseudopregnant rats [28, 29]. Contrary to testosterone, progesterone is not reduced in its 5α-position in order to exert its biological action, a fact also confirmed by studies of Wiest and Rao[10] on the competition of pregnane steroid with progesterone for binding sites on proteins of rabbit uterine and human endometrium cytosol: the binding index of 5α-pregnane 3,20-dione is 7% of that found for progesterone in the human endometrium, whereas values under 2% were found for 20β-(and 20α-)hydroxy-4-pregnen-3-one and 5β-pregnane-3,20-dione.

This report allows the conclusion that the human myometrium, as the uterus of other mammalian species (cf. [30]) metabolizes progesterone to form several inactive compounds. It was not possible in this investigation and those of others to detect a 5β-ring reductase activity in the human uterus. To this extent the metabolism of testosterone in the human prostate is similar to that of progesterone in the human myometrium.

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