Journal of Steroid Biochemistry, 1975, Vol. 6. pp. 1131–1136. Pergamon Press. Printed in Great Britain

METABOLISM OF OESTRADIOL-17β IN THE HUMAN MYOMETRIUM

B. P. LISBOA and H.-D. SAUER*

Universitäts-Frauenklinik Eppendorf, Martinistraße 52, 2 Hamburg 20, Germany

(*Received* 15 *February* 1974)

SUMMARY

The metabolism of 4-^{14}C-oestradiol-17β in preparations of human myometrium was investigated. The steroid tissue ratio was between 10^3 and 10^6. Minced uterus, brei and homogenate preparations were incubated with oestradiol at 37°C for a period of 30–90 min with and without addition of cofactors.

After purification by solvent partition in methanol–petroleum–ether–water the steroids were isolated by column gel chromatography on Sephadex LH-20. The metabolites were identified by their behaviour on gel column, thin-layer and gas chromatography, by radio gas chromatography and by calculation of constant activity in eluates obtained from gel column chromatography on Sephadex LH-20.

Under all the experimental conditions employed oestradiol-17β was metabolized to oestrone, 16-epi-oestriol, oestriol, 16α-hydroxyoestrone and 16-keto-oestradiol.

INTRODUCTION

Since oestrogen receptors in the human uterine tissue have been demonstrated by Wyss *et al.*[1], Gabb and Stone[2] and Mešter *et al.*[3] the metabolism of oestrogens in this tissue has been of particular interest.

Hähnel *et al.*[4] studied the specificity of the oestrogen receptor of human uterine tissue. Their results indicate that the oestradiol binding capacity of the receptor is strongly affected by several possible oestradiol metabolites, as for instance oestrone, oestriol, 16-epi-oestriol and 17-epi-oestriol.

The purpose of this study was to investigate the metabolism of oestradiol-17β in the human myometrium, and whether there could be a formation in this tissue of metabolites which could compete with oestradiol-17β for binding sites in the uterine receptor.

MATERIAL AND METHODS

Steroids

The non labelled steroids were obtained from the following sources: Oestrone (3-hydroxy-1,3,5(10)-oestratrien-17-one), oestradiol-17β (1,3,5(10)-oestratrien-3β,17β-diol), 16α-hydroxyoestrone (3,16α-dihydroxy-1,3,5(10)-oestratrien-17-one), and oestriol (1,3,5(10)-oestratriene-3,16α,17β-triol) were from Merck A. G., Darmstadt, Germany; and 16-oxo-oestradiol-17β (3,17β-dihydroxy-1,3,5(10)-oestratrien-16-one) and 16-epi-oestriol (1,3,5(10)-oestratriene-3,16β,17β-triol) were from Steraloids Inc. Pawling, U.S.A. The purity of these steroids was assayed by thin-layer and gas–liquid chromatography.

Labelled [4-^{14}C]-oestradiol-17β was purchased from New England Nuclear Chemicals Gmbh, Dreieichenhain, Germany (Article n. NEC-127) and had a specific activity of 58·2 mCi/mmol. The purity of this compound was assayed by radio-gas–liquid chromatography.

Non steroids

dl-Isocitrate trisodic 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. All other reagents and solvents were purchased from Merck A. G., Darmstadt, Germany, and were of highest purity (analytical grade).

Chromatographic techniques

(a) *Thin-layer chromatography.* Analytical one-dimensional ascending thin-layer chromatography (t.l.c.) was carried out on 20 × 20 cm precoated silica gel layers (silica gel 60 Merck, Darmstadt, 0·25 mm) according to the specifications of Lisboa[5, 6], using the solvent system cyclohexane–ethylacetate–ethanol 45:45:10 by vol. Authentic standards running simultaneously with the extracts were developed with the Folin–Ciocalteu or with the anisaldehyde–sulphuric acid–acetic acid reagents [7]. Radiochromatograms were scanned by a thin-layer-scanner with methan flow (Labor. Prof. Berthold, Wildbad, Germany), model IILB 2722, with counting tube LB 2007/LB 6280. Labelled material was extracted from the layers by elution with absolute ethanol through a synthesized glass[8].

Oestrogens were detected in a non destructive procedure by reflectance spectrometry at 281 nm on a Zeiss-chromatogram spectralphotometer (Carl Zeiss, Oberkochem, Germany)

* Taken in part from a dissertation by H.-D. Sauer in partial fulfilment of the requirements for the M.D. degree, Medical School, University of Hamburg.

(b) *Gel chromatography on Sephadex.* Column gel chromatography (c.g.c.) on Sephadex LH-20 (particle size 25–100 μm, Pharmacia, Fine Chemicals, Sweden) was carried out on 1 × 30 cm glass columns by using solvent systems [7, 9, 10] *S-1* n-heptane–chloroform–methanol 80:10:10 by vol. *S-2* iso-octane–chloroform–methanol 80:10:10 by vol., *S-3* n-heptane–chloroform–methanol 88:10:2 by vol. and *S-4* cyclohexane–chloroform–methanol 80:10:10 by vol.

Gel chromatography of labelled metabolites and authentic standards was used for identification purposes, when a constant specific activity was found in the different eluates corresponding to the isolated material. Specific activity in the eluates obtained from gel column chromatography was calculated by simultaneous measurement of radioactivity and added cold standard in each eluate. Cold oestrogens were determined by using the Kober-reaction modified according to Bauld[11]. Radioactivity was measured in a Packard liquid scintillation spectrometer (Model 3380) after addition of 10 ml Instagel (Packard, U.S.A.) to the glass vials containing the dried aliquots.

(c) *Gas–liquid chromatography.* Gas chromatographic analysis was carried out on a gas chromatograph Pye series 104 (Pye Unicam Ltd. England) using 270 cm × 4 mm spiral glass columns with 2·2% silicon GE SE-30 on Gaschrom Q 100/120 μm or with 3% silicon QF-1 on gaschrom Q 125/150 μm (phases and support obtained from W. Günther Analysentechnik, Düsseldorf, Germany). Temperature of the column, injectionblock and flame ionization detectors were 225, 255 and 300°C respectively. Gas pressure of hydrogen and synthetic air was 1·2 kg/cm^2 for both. Purified nitrogen was employed as carrier (20–30 ml/min). Steroids were chromatographed as trimethyl silyl ether derivatives (TMSi) prepared according to Makita and Wells[12], or after methoxime formation according to Gardner and Horning[13].

(d) *Radio gas chromatography.* A Pye gas chromatograph associated with the detector assembly for radio gas chromatography manufactured by Panax Equipment Ltd., England, was employed. The splitter between FID and radio section was 1:1. The temperature of the flowing reactor was 650°C. Argon was used as gas carrier (48 ml/min) and carbondioxide (2 ml/min) as quenchgas. The furnace tube was filled with cupric oxide.

Myometrium preparations

Uterine tissue was obtained from patients 40–45 years old undergoing hysterectomy for prolapse. Myometrium obtained immediately after uterus extirpation was separated from endometrium, washed in physiological sodium chloride solution and minced into small portions, which were employed for the preparation of myometrium brei or 10% homogenate preparations. Only the macroscopically normal parts of the uteri were minced. The myometrium brei was prepared by homogenizing 4–10 g minced tissue at 4°C in an Ultra Turrax apparatus with a Krebs–Ringer—0·25 mol/l-glucose solution to a final volume of 30 ml. A 10% homogenate suspension was prepared from a myometrium brei in 0·25 mol/l sucrose solution using a Potter–Elvehjem homogenizer with Teflon pestle at a speed of 1500 rev./min.

Conditions of incubation

Different sets of incubations were carried out with minced myometrium (I), myometrium brei (II) and with a homogenate preparation (III).

(I) Incubation with minced myometrium. Minced myometrium corresponding to 5 g tissue was incubated with 1 μCi [4-^{14}C]-oestradiol without additions of cofactors at 37°C for 30 min in 30 ml of a Krebs–Ringer glucose solution (containing 20 mmol/l glucose); the pH of the incubation medium was 6·8 (ratio steroid–tissue = 1:1,087,000).

(II) Incubation was myometrium brei. Three different sets of incubations were carried out with the brei preparation. (IIa) and (IIb): A myometrium preparation containing 5 g tissue in 15 ml Krebs–Ringer glucose solution together with a NADPH-regenerating system (1 ml 0·2 mol/l NADP, 1 ml 0·05 mol/l sodium isocitrate, 1 ml 0·2% isocitrate dehydrogenase) and manganesedichloride (1 ml of 0·1 mmol solution) was incubated (pH 6·8) at 37°C for 60 min with (IIa) 5 mg oestradiol (dissolved in propyleneglycol) or (IIb) 1 μCi [4-^{14}C]-oestradiol and 5 mg oestradiol (ratio steroid–tissue = 1:1000). (IIc) A brei preparation containing 2 g tissue in 15 ml Krebs–Ringer glucose solution, 1 ml of 0·1 mmol/l $MnCl_2$ solution, and 1 ml of Krebs–Ringer glucose solution (containing 1 μCi oestradiol) was incubated at 37°C for 60 min without addition of cofactors (pH 6·8). This incubation (IIc) was used for quantitative determination of the radioactive metabolites derived from oestradiol at low concentrations (ratio steroid–tissue = 1:438,600).

(III) Incubation with a 10% homogenate preparation. One μCi of [4-^{14}C]-oestradiol-17β and 2 mg oestradiol-17β (dissolved in propyleneglycol) were incubated with 20 ml of a 10% homogenate preparation (in 0·25 mol/l sucrose solution) 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, 1 ml 0·02 mol/l NADH, and a NADH-regenerating system (4 ml 0·05 mol/l natrium isocitrate–3 ml 0·02 mol/l NADP–0·4 ml of a 10% isocitratedehydrogenase solution at 37°C for 90 min (ratio steroid–tissue = 1:2500). In all incubation sets the enzymatic reaction was stopped by addition of 10 ml of a mixture of ether–chloroform 3:1.

Purification of extracts

All the incubations were extracted three times with ether–chloroform 3:2 v/v; the incubation phase was centrifugated and the sediment, after ultrasound treatment, was extracted successively with 50 ml chloroform and methanol. The five organic extracts were pooled, evaporated and purified according to the method of Fukayama *et al.*[14]. The pooled ethylacetate phase contained between 98 and 99% of the total radioactivity. In each of the two other phases—petro-

leum ether and water—less than 1% radioactivity was found; a preliminary analysis of the petroleum ether phase has shown that the radioactivity was identical to the incubated steroid and to a less polar compound (identified as oestrone) in a pattern similar to that found in the ethylacetate phase. Therefore only the ethylacetate phase was employed for further work.

RESULTS

(a) *Incubation with minced myometrium* (I)

Aliquots of the ethylacetate fraction of incubation carried out with minced myometrium (I) were subjected to t.l.c. in the solvent system ethyl acetate–cyclohexane–ethanol 45:45:10 by vol. and gel column chromatography on 4 g Sephadex LH-20, system S-1, flow rate 0·5–0·6 ml/min. The t.l.c. has shown radioactive peaks isopolar to oestrone, 16-epi-oestriol and oestriol besides large amounts of radioactive material in the region of oestradiol-17β.

The gel chromatogram had five definite regions corresponding to the fractions: (a) between 30 and 45 ml, i.e. the elution volume of oestrone (27·5–47·5 ml); (b) between 45 and 100 ml, that is to unmetabolized oestradiol (60–90 ml); (c) between 100 and 120 ml, isopolar with 16-oxo-oestradiol or 16α-hydroxyoestrone (110–135 ml); (d) between 125 and 150 ml, isopolar with 16-epi-oestriol (120–160 ml), and (e) 175–200 ml, isopolar with oestriol (175–220 ml).

(b) *Incubations with myometrium brei* (IIa, IIb *and* IIc)

The ethylacetate extracts of the incubations IIa, IIb and IIc carried out with myometrium brei were subjected to gel column chromatography on 4 g Sephadex LH-20 using 220 ml of system S-1. Fractions corresponding to 5 ml eluates were collected, evaporated and analysed by gas chromatography (incubation IIa) or its radioactivity was measured in a scintillation counting spectrometer (IIb, IIc). Gas chromatographic analysis of the fractions collected from the experiment IIa (without radioactivity) were done after methoxime (Mo-) or silylether (TMSi–ether) formation in two chromatographic phases, SE-30 and QF-1. In the fractions corresponding to 30–40 ml oestrone was identified as TMSi–ether, and Mo-derivatives in the two phases as indicated in Table 1. A typical gas chromatogram of the isolated oestrone TMSi–ether on a 2·2 per cent SE-30 column is shown in Fig. 1. In the fraction 90–110 ml, besides huge amounts of oestradiol, two peaks with the rrt-values of 16α-hydroxy-oestrone (1·82) and 16-oxo-oestradiol (very small amounts 2·36) were found on QF-1-columns after TMSi–ether formation. A peak with the rrt of 16α-hydroxy–oestrone TMSi–ether was also found in the fraction 110–120 ml, whereas in the 120–150 ml fraction a peak with similar rrt-value to the TMSi–ether of 16-epi-oestriol was detected on both phases (QF-1 1·51; SE-30 1·32). Finally, small peaks with the rrt of oestriol TMSi–ether were found in a pooled fraction corresponding to 190–250 ml of the eluate (QF-1: isolated material 1·37, standard 1·39; SE-30: isolated material 1·29, standard 1·28).

Table 1. Relative retention times (5α-cholestane = 1·0) of the TMSi–ether and methoxime derivatives of a compound isopolar to oestrone isolated from the incubation of oestradiol with brei preparations of human myometrium chromatographed on a 3% QF-1 and 2·2% SE-30 phases at 225°C. For comparison the values found for the TMSi–ethers of oestrone and oestradiol and oestrone–methoxime under the same experimental conditions are given

	TMSi–ether		Methoxime	
	QF-1	SE-30	QF-1	SE-30
Isolated compound	0·53	1·82	1·19	0·57
Oestrone	0·53	1·82	1·19	0·56
Oestradiol	0·85	0·65	—	—

The column radio chromatogram of an aliquot of the purified extracts of the incubation carried out with 1 μCi and 5 mg oestradiol obtained in system S-2 is reproduced in Fig. 2.

This chromatogram was quite similar to that found in the incubation carried out with minced myometrium and equally had five zones with the following distribution of radioactivity (in % of total radioactivity): (a) fractions 12–20, 14,630 c.p.m., 2·36%, isopolar with oestrone; (b) fractions 21–42, 584,146 c.p.m., 94·19%, corresponding to nonmetabolized oestradiol; (c) fractions 43–51, 6269 c.p.m., isopolar with 16-oxo-oestradiolor 16α-hydroxy-oestrone; (d) fractions 52–62, 6450 c.p.m., 1·04%, isopolar with 16-epi-oestriol and (e) fractions 63–78, 4630 c.p.m., 0·76%, isopolar with oestriol. Further identification of these metabolites was carried out by radio gas chromatography.

The third incubation of this series with 1 μCi of oestradiol (4·54 μg) was analysed under the same conditions as IIb; about 82% of oestradiol remained unmetabolized (peak b) whereas the radioactivity found for

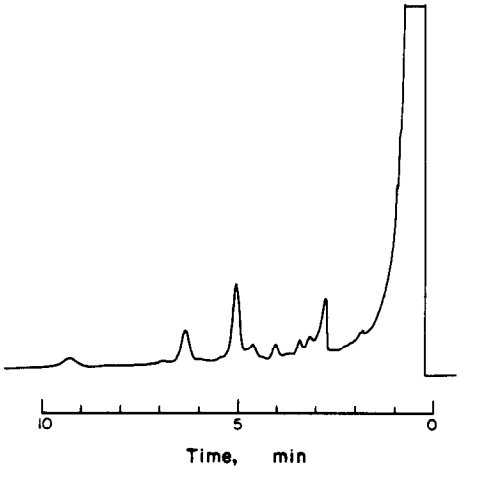

Fig. 1. Gas–liquid chromatography of the fraction 30–40 ml obtained from gel column chromatography on Sephadex LH-20, developed with solvent system S-1, of the ethylacetate extract obtained after incubation of unlabelled oestradiol with a brei preparation of human myometrium. After silyl-ether derivative formation the gas chromatogram was obtained on a 2·2% SE-30 column, and the peak with a rrt to cholestane of 1·82 is isopolar to the oestrone–silyl-ether.

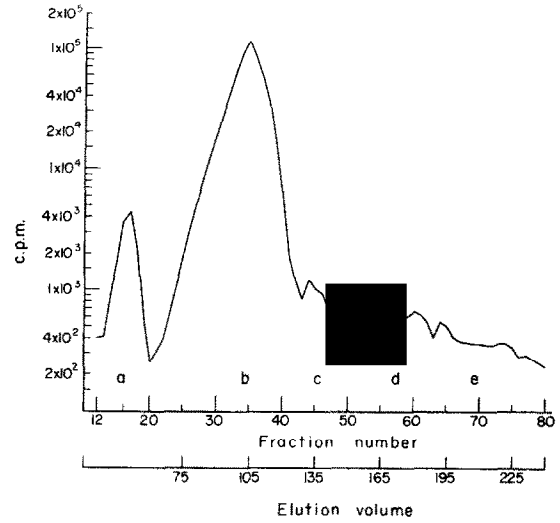

Fig. 2. Gel column chromatography on 4 g Sephadex LH-20 with solvent system S-2 of aliquots of the ethylacetate extract obtained after the incubation of 1 μCi and 5 mg oestradiol with myometrium brei preparation corresponding to 5 g tissue. Radioactivity was determined by liquid scintillation counting. The radioactive zones were isopolar with the following oestrogens: (a) oestrone; (b) oestradiol; (c) 16-oxo-oestradiol and 16α-hydroxyoestrone; (d) 16-epi-oestriol; and (e) oestriol.

oestrone and for the $C_{18}O_3$ steroids amounted to 10·5 and 4·0% of total radioactivity. In this experiment oestrone was identified by gel column chromatography of the material isolated in peak *a* (30–45 ml eluate) together with 300 μg of authentic oestrone on a 4 g Sephadex LH-20 column (1 × 11·5 cm) in the solvent system S-3; aliquots of 8 ml fractions collected with a flow-rate of 0·7 ml/min were analysed for radioactivity (in a scintillation spectrometer). The amount of oestrone in each fraction was measured by a colourimetric procedure in which the sulfuric acid chromogen developed by the Kober-reaction [7] is measured at 512·5 nm. As shown in Fig. 3 a constancy of the specific activity was found in all the fractions collected between 112 and 168 ml.

16α-Hydroxy-oestrone was identified by radio gas chromatography in the material isolated from peak *c*; besides oestradiol-17β a radio peak was found with the same rrt of authentic 16α-hydroxy-oestrone-TMSi-ether (rrt to oestradiol-17β: 2·34). Oestriol and 16α-epi-oestriol were identified as TMSi-ether derivatives in a pool of the fractions eluated between 135 and 220 ml, by radio gas chromatography (Fig. 4).

(c) *Incubation with homogenate preparations* (III)

Aliquots of the ethylacetate extract from the incubation III were investigated by t.l.c. and g.l.c. The silica gel chromatogram developed in the solvent system cyclohexane–ethylacetate–ethanol 45:45:10 (by vol.) (Fig. 5) showed the presence of two $C_{18}O_3$ compounds isopolar to oestriol (3 cm) and 16-epi-oestriol (4·5 cm; front: 15·5 cm); ketolic oestrogens and oestrone remained unresolved from oestradiol (*Rs* values to oestradiol: oestrone 1·13, 16α-hydroxy-oestrone 0·93, 16-oxo-oestradiol, 0·98).

The column radio chromatogram on Sephadex LH-20 (system S-1) was quite identical to those obtained from the previous incubations I and II. Oestrone was identified in the fraction 30–45 ml by radiogas chromatography on a SE-30 column after TMSi-ether formation. Oestriol and 16-epi-oestriol have been identified in the fraction 110–210 ml corresponding to $C_{18}O_3$ steroids; together with 300 μg oestriol and 250

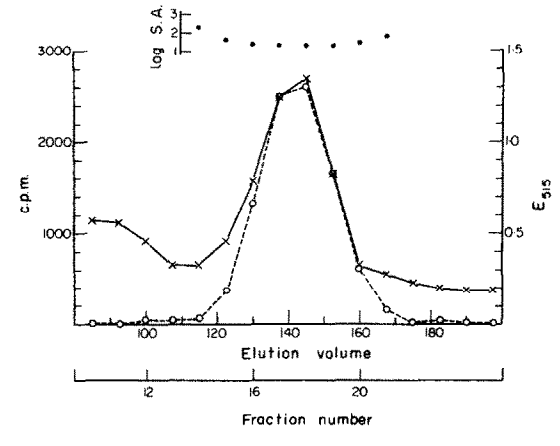

Fig. 3. Column radio chromatogram of the radioactive material isolated from the fraction 30–45 ml of the Sephadex chromatogram of the incubation of [^{14}C]-oestrone with myometrium brei preparations, to which 300 μg of authentic oestrone was added. Gel chromatography developed on 4 g Sephadex LH-20 with solvent system S-3; specific activity in each 8 ml fraction was calculated by measuring the radioactivity in a liquid scintillation counting apparatus, and measuring the steroid weight expressed as absorption value of its sulfuric acid chromogenicity (Kober reaction) assessed by spectrophotometry at 512·5 nm.

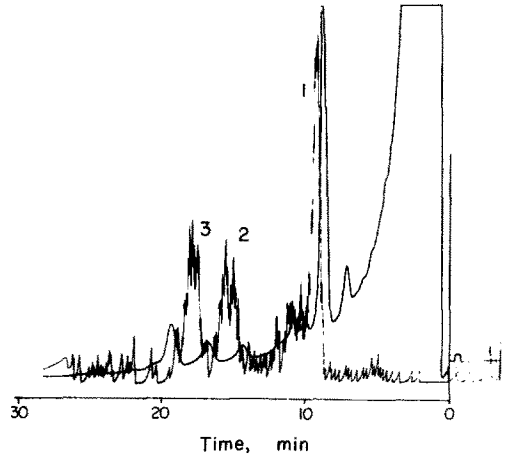

Fig. 4. Radio gas–liquid chromatography of the fraction 135–220 ml obtained from gel column chromatography (on Sephadex LH-20 in the solvent system S-3) of the extract obtained after incubation labelled oestradiol with a brei preparation of human myometrium. The radio gas chromatogram was obtained after TMSi-ether derivative formation in a 3% QF-1 phase; paper speed 1 cm/min, ratio labelled/nonlabelled compound: 1·06. Oestriol and 16-epi-oestriol (as TMSi-ethers) were chromatographed simultaneously. Radioactive peaks (1), (2) and (3) correspond to oestradiol, oestriol and 16-epi-oestriol, respectively.

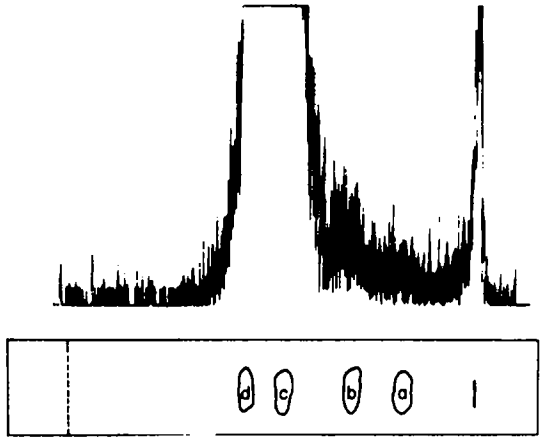

Fig. 5. Ascending thin-layer chromatogram of an aliquot of the extract obtained from the incubation of [^{14}C]-oestradiol with a homogenate preparation of human myometrium, on silica gel layer developed with cyclohexane–ethylacetate–ethanol 45:45:10 by vol. The layer was scanned on a radio scanner (measure range 3 c.p.s.; time constant 3 sec, paper speed 120 mm/h). For comparison, a chromatogram of authentic standards which were running simultaneously: (a) oestriol; (b) 16-epi-oestriol; (c) oestradiol; and (d) oestrone.

μg 16-epi-oestriol this fraction was rechromatographed on 4 g Sephadex columns (1 × 12·5 cm column; flow rate 0·5–0·7 ml/min; 4 ml fraction) developed with system S-4. Figure 6 shows a complete identity of the specific activities measured for both steroids in all the chromatographed fractions. A third radioactive peak in this radio chromatogram probably corresponds to 16α-hydroxy-oestrone (60–90 ml) and/or 16-oxo-oestradiol (70–95 ml).

DISCUSSION

Contradictory results concerning the biotransformation of oestradiol to oestrone in the mammalian uterus have been reported in the last years. According to investigations of Szego and Samuels[15] and Abe *et al.*[16] there is no evidence for the conversion of oestradiol to oestrone in human uterine tissue. In addition no metabolism of oestradiol was found in experiments carried out with the bovine endometrium [15] or immature rat uteri [17]. In contrast, Ryan and Engel[18] observed conversion of oestradiol to oestrone by human hyperplastic endometrium and fibroid myometrium slices in a yield of 0·5 to 1·7%. This was confirmed by Sweat *et al.*[19] and Collins *et al.*[20] investigating the interconversion between oestradiol and oestrone in the human endometrium and myometrium. Lehmann and Breuer[21] reported similar conversions in the rat myometrium, Jütting *et al.*[22] in rabbit myometrium preparations and Russell and Thomas[23] in cultured rabbit uterus.

In the present investigation the isolation and characterization of oestrone, 16-epi-oestriol and oestriol after incubation of oestradiol-17β with homogenate preparations of human myometrium has been achieved. Two further metabolites detected showed the chromatographic behaviour of 16-oxo-oestradiol-17β and 16α-hydroxy-oestrone.

The isolation of oestrone and detection of 16α-hydroxy-oestrone indicates the presence of a 17β-hydroxysteroid dehydrogenase in the uterus and confirms the previous observations mentioned above on the interconversion between oestrone and oestradiol in human myometrium. Under our experimental conditions where 1 μCi (4·54 μg) oestradiol had been incubated for 60 min. with preparations corresponding to 2 g tissue,

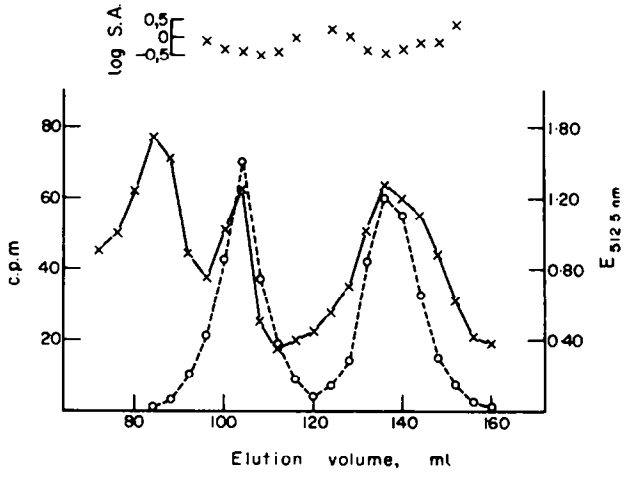

Fig. 6. Gel column chromatography on 4 g Sephadex LH-20 developed in solvent system S-3 for the identification of two $C_{18}O_4$-metabolites which were isolated in the polar fraction (110–210 ml) obtained after chromatography on Sephadex (S-4) of the incubation of [^{14}C]-oestradiol with homogenate preparation of myometrium. This chromatogram on a 1 × 12·5 cm column with a flow rate of 0·5–0·7 ml/min was developed after 300 μg oestriol and 250 μg 16-epi-oestriol had been added to the polar fraction. Specific activity (S.A.) for all 4 ml fractions between 72 and 164 ml was calculated by comparing the radioactivity measured on a liquid scintillation counting apparatus with the weight expressed as the absorption values of the sulfuric acid chromogenicity developed by the added standards measured at 512·5 nm. Oestriol: 120–160 ml; 16-epi-oestriol: 84–120 ml.

10·5% of the total radioactivity recovered was isolated and identified as oestrone. The lack of oestrone formation described by other authors cannot be explained by a low yield in this transformation, but by their different experimental conditions and/or less sensitive methods employed in the early years.

It should be pointed out that in our experiments the metabolization of oestradiol in the human myometrium occurred at low concentrations of 4·54 μg steroid for 2 g tissue during 60 min. (36·7 pg/mg/min). This is only the 3·5-fold concentration of *in vivo* studies on the uptake of oestradiol by the uterus of rodents (1 μg/100 g of body weight, cf. Tchernitchin and Chandross[24]. In the human endometrium, Sweat and Young[25] demonstrated the transformation of oestradiol to oestrone at much lower concentrations (2 fg/min/mg tissue).

The isolation of 16-oxygenated compounds in this investigation demonstrates the presence of a 16α-hydroxysteroid-oxidoreductase in the human myometrium responsible for the formation of oestriol and of 16α-hydroxy-oestrone. The formation of 16-epi-oestriol could be explained by either a 16β-hydroxylase or a 16α (and 16β)-hydroxysteroid-oxidoreductase in the human momentum.

The evidence for oestrone and 16-oxygenated oestrogens to be uterine metabolites of oestradiol is of great importance since oestrone, and to a much lesser extent the 16-oxygenated oestrogens, induce the uterine growth, as demonstrated by Huggins and Jensen[26] by *in vivo* experiments in the rat.

Furthermore three of the metabolites isolated in this study—oestrone, 16-epi-oestriol and oestriol—compete very strongly with oestradiol for binding sites at the oestrogen receptor of human uterus. Hähnel and coworkers [4] observed that the affinity of oestradiol to its receptor was decreased to 40 or 50% of the control value by 1 ng of oestriol or 16-epi-oestriol and oestrone, respectively; similar effects have been shown for higher concentrations of 16,17-ketolic oestrogens.

Acknowledgement—This investigation was supported by the Deutsche Forschungsgemeinschaft (Sonderforschungsbereich Endokrinologie 34).

REFERENCES

1. Wyss R. H., Karsznia R., Heinrichs W. L. and Herrmann W. L.: *J. clin. Endocr. Metab.* **28** (1968) 1824–1828.
2. Gabb R. G. and Stone G. W.: *Abstracts 12th Gen. Meeting Endocrine Society*, Australia, Brisbane (1969) 12.
3. Mešter J., Robertson D. M., Feherty P. and Kellie A. E.: *Biochem. J.* **120** (1970) 831–836.
4. Hähnel R., Twaddle E. and Ratajczak T.: *J. steroid Biochem.* **4** (1973) 21–31.
5. Lisboa B. P.: In *Steroids and Terpenoids* (Edited by Clayton R.), *Methods in Enzymology* XV. Academic Press, New York (1968) 3–158.
6. Lisboa B. P.: *Clin. chim. Acta* **13** (1966) 179–199.
7. Lisboa B. P.: In *Fundamentals of Lipid Chemistry* (Edited by Burton R. M. and Guerra F. C.). BI-Science Publications, Webster Groves, Missouri, U.S.A. (1974) pp. 439–473.
8. Böttinger L. E. and Lisboa B. P.: *Z. Klin. chem. Klin. Biochem.* **5** (1967) 176–182.
9. Lisboa B. P. and Strassner M.: *VII Int. Symposium Chromatography Electrophoresis*, Brussels, 1972. Academic Press Europ. (1973) 205–220.
10. Lisboa B. P. and Strassner M.: unpublished results.
11. Bauld W. S.: *Biochem. J.* **56** (1954) 426–434.
12. Makita M. and Wells W. W.: *Analyt. Biochem.* **5** (1963) 523–530.
13. Gardiner W. L. and Horning E. C.: *Biochem. biophys. Acta* **115** (1966) 524–526.
14. Fukayama G. M., Barrett C., Wood P. D. S. and Crowley L. G.: *J. clin. Endocr. Metab.* **33** (1971) 677–682.
15. Szego C. M. and Samuels L. T.: *J. biol. Chem.* **151** (1943) 599–605.
16. Abe T., Hagerman D. D. and Villee C. A.: *J. biol. Chem.* **239** (1964) 414–418.
17. Jensen E. V. and Jacobson H. I.: *Recent Prog. Horm. Res.* **18** (1962) 387–414.
18. Ryan K. J. and Engel L. L.: *Endocrinology* **52** (1953) 287–291.
19. Sweat M. L., Bryson M. J. and Young R. B.: *Endocrinology* **81** (1967) 167–172.
20. Collins W. P., Mansfield M. D., Bridges C. E. and Sommerville I. F.: *Biochem. J.* **113** (1969) 399–407.
21. Lehmann W. D. and Breuer H.: *Naturwissenschaften* **55** (1968) 182–183.
22. Jütting G., Thun K. J. and Kuss E.: *Eur. J. Biochem.* **2** (1967) 146–151.
23. Russell S. L. and Thomas G. H.: *J. Endocr.* **53** (1972) xxix.
24. Tchernitchin A. and Chandross R.: *J. steroid Biochem.* **4** (1973) 41–44.
25. Sweat M. L. and Yound R. B.: *Biochim. biophys. Acta* **296** (1973) 189–195.
26. Huggins C. and Jensen E. V.: *J. exp. Med.* **102** (1955) 335–346.