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17β-HYDROXYSTEROID DEHYDROGENASE ACTIVITY IN LEIOMYOMA AND MYOMETRIUM AND ITS RELATIONSHIP TO CONCENTRATIONS OF OESTRONE, OESTRADIOL AND PROGESTERONE THROUGHOUT THE MENSTRUAL CYCLE

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Summary—The activity of the enzyme 17β-hydroxysteroid dehydrogenase (17β-OHSD) and concentrations of oestrone (E_1), oestradiol (E_2) and progesterone have been measured in leiomyoma and myometrium obtained at different stages of the menstrual cycle. Apart from conversion of E_2 to E_1 in the proliferative phase, no significant difference in enzyme activity was noted between normal and tumour tissue. However, interconversion in both tissues was shown to be higher in the secretory than the proliferative phase of the menstrual cycle. E_1 concentrations were significantly higher ($P < 0.01$) in leiomyoma than in myometrium, obtained during the proliferative phase. Concentrations of both oestrogens, in some tumour and normal tissues, were higher in the proliferative than the secretory phase. Secretory phase tissues contained higher concentrations of progesterone than those obtained in the proliferative phase of the menstrual cycle. Considerable differences in both enzyme activity and steroid concentrations were noted in different areas of the same tumour.

INTRODUCTION

The interconversion of oestrone (E_1) and oestradiol (E_2) in various body tissues is mediated by the enzyme 17β-hydroxysteroid dehydrogenase (17β-OHSD). Kinetic studies of the enzyme in endometrial tissue indicate that oxidation, rather than reduction is favoured [1]. Tseng and Gurpide [2] have shown that there is a sharp rise in endometrial 17β-OHSD activity at the onset of the secretory phase of the menstrual cycle, and *in vitro* studies performed by the same workers have demonstrated that progesterone can induce the synthesis of this enzyme [3]. It is therefore likely that at the onset of the secretory phase of the menstrual cycle when tissue progesterone concentrations are rising, progesterone acts via a receptor mechanism to induce synthesis of the dehydrogenase. This mechanism may have evolved to limit the growth-stimulating effect of oestradiol during the secretory phase, when plasma oestradiol concentrations are still relatively high.

Several studies have been performed to measure the activity of 17β-OHSD in myometrium [4–7]. Not all of these show that enzyme activity rises during the secretory phase of the menstrual cycle [4, 5]. Schmidt-Gollwitzer *et al.* [6] found that enzyme activity in myometrium as in endometrium rises during the early secretory phase in parallel with tissue progesterone. Pollow *et al.* [7] have provided evidence that the oxidation of oestradiol in myometrium and leiomyoma tissue is greater in the secretory than the proliferative phase, although 17β-OHSD activity in tumour tissue was considerably lower than in myometrium. The explanation put forward was that the protective mechanism to limit tissue exposure to oestradiol is altered in leiomyoma tissue.

Recent *in vitro* studies from this laboratory suggest that some leiomyomas have the ability to aromatise androstenedione [8]. It was proposed that localised production of oestrone is an important factor in the growth of some of these tumours. However, since oestradiol is the active oestrogen, further reduction of oestrone to oestradiol is a necessary step in the production of active oestrogen from androgen. The following study was undertaken to examine the activity of 17β-OHSD, and to consider its role in determining steroid concentrations in normal myometrium and leiomyoma throughout the menstrual cycle.

EXPERIMENTAL

Clinical material

Myometrium and leiomyoma were obtained at hysterectomy from 20 premenopausal patients aged between 30 and 52 years. Tissue was immediately transported on ice to the laboratory for storage at −20°C until required for assay. One postmenopausal subject receiving steroid replacement therapy for symptoms of the menopause was included in this study.

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Chemicals

[4-^{14}C]Oestrone (55 mCi/mmol), [2,4,6,7-^{3}H]-oestradiol (93 Ci/mmol), [2,4,6,7-^{3}H]oestrone (86 Ci/mmol), [2,4,6,7-^{3}H]oestradiol (86.8 Ci/mmol) and [1,2,6,7-^{3}H]progesterone (87 Ci/mmol) were purchased from Amersham International PLC, U.K. Purity was checked by thin layer chromatography (TLC). Unlabelled steroids and cofactors were obtained from the Sigma Chemical Company.

Tissue preparation

Tissue was rinsed with 0.1 M phosphate buffer pH 7.4, minced finely, and weighed. Homogenisation was performed using a Polytron homogeniser in 25–50 vol of buffer. An aliquot of homogenate was treated with collagenase as previously described [8] and stored at −20°C for the measurement of tissue steroid concentrations. The remainder was used to determine enzyme activity.

Enzyme activity

The activity of 17β-OHSD was investigated in both tumour and normal myometrium by the use of a double isotope method similar to that previously described [9, 10]. Under conditions chosen to measure the initial velocity of reaction, the operation of the back reaction for both directions was negligible (0.5% of the forward reaction for $E_1 \rightarrow E_2$ and 0.1% of the forward reaction for $E_2 \rightarrow E_1$).

Incubation procedure

Reaction mixtures to measure the conversion of oestrone to oestradiol contained 1 ml of tissue homogenate (24–40 mg tissue wet wt/ml buffer), [4-^{14}C]oestrone (140,000 cpm–2414 pmol), glucose-6-phosphate (10 μmol), NAD (2 μmol) NADP (2 μmol) and [2,4,6,7-^{3}H]oestradiol (35,000 cpm) to monitor procedural losses. Production rates of oestrone were determined following the addition of [2,4,6,7-^{3}H]oestradiol (100,000 cpm), 1080 pmol oestradiol, NAD (2 μmol) and [4^{14}C]oestrone (3000 cpm) to a further 1 ml aliquot of tissue homogenate. Reactions were started by the addition of glucose-6-phosphate dehydrogenase and NAD respectively. Incubations were carried out at 37°C in a shaking water bath for 1 h. and the reactions were terminated by the addition of methanol to give a 50% aqueous solution.

Extraction and separation of products

The incubation medium was extracted with 5 ml of diethyl ether and oestrone and oestradiol were separated by TLC on silica gel plates (E. Merck, Darmstadt, Germany) using the system dichloromethane–ethyl acetate (80:20, v/v). The separated steroids were then located by a radiochromatogram scanner (Panax), the appropriate areas eluted and radioactivity measured in a Beckman LS 7500 liquid scintillation counter. The isotope ratios of the isolated products was determined and this ratio was used to calculate the amount of product formed according to the following formula:

$$\text{product (pmol)} = \frac{\text{cpm product/cpm recovery} \times \text{recovery cpm added}}{\text{specific activity substrate (cpm/mol)}}$$

The consistency of isotope ratios obtained by this method was verified by two further TLC systems: cylcohexane–ethyl acetate (1:1, v/v) and toluene–ethyl acetate (60:40, v/v). A single system was used in subsequent assays.

Radioimmunoassay of oestrogens and progesterone in tissue

Tissue concentrations of oestrone, oestradiol and progesterone were measured according to the method previously described for plasma [11]. Duplicate 0.75 ml aliquots of collagenase treated homogenates were used for all assays. Tissue concentrations of oestrone were measured without prior purification using TLC as no significant difference in oestrone concentration could be demonstrated with and without TLC ($t = 0.18$, $df = 18$). However, oestradiol and progesterone concentrations were determined following separation by TLC, using the solvent system dichloromethane–ethyl acetate (90:10, v/v). For each steroid measured the intra-assay coefficient of variation, cold recovery and linearity over a tissue concentration range of 10–60 mg/0.75 ml is summarised in Table 1.

RESULTS

Substrate and cofactor requirements

Over a range of substrate concentrations (E_1, 3.0–35 μM, E_2, 0.6–18 μM) the apparent Michaelis–Menton constant (K_m) and maximum velocity (V_{max}) was determined for both forward and reverse reac-

Table 1. Summary of data obtained for steroid assay validation

	Oestrone				Oestradiol				Progesterone			
Intra assay CV (%)	6.4				12.9				10.8			
$x \pm$ SD (pg/ml) $n = 10$	42.5 ± 2.7				52.8 ± 6.8				98.6 ± 10.7			
Linearity $y =$	0.41x + 1.88				0.75x − 2.32				1.36x + 7.82			
Correlation coefficient	0.99				0.97				0.99			
Recovery												
Steroid added (pg)	7,	15,	30,	60	7,	15,	30,	60	10,	20,	40,	80, 160
Steroid recovery (pg)	7.3	16,	33,	77	5.7,	12.9,	30,	65	11.6,	22.5,	48.4,	89.4, 150.9

Table 2.

	$E_1 \rightarrow E_2$	$E_2 \rightarrow E_1$
K_m (μM)		
L	15.9 ± 7.8	2.0 ± 0.2
M	52.8 ± 41.4	3.4 ± 0.9
V_{max}*		
L	9.4 ± 2.1	2.2 ± 0.5
M	79.4 ± 65.5	2.1 ± 0.4

*nmol/g tissue/h.

Apparent Michaelis–Menton constant (K_m) and maximum velocity (V_{max}) of samples of myometrium (M) and leiomyoma (L), as determined by the method Lineweaver and Burk. Values are expressed as mean and standard deviation of three tissues.

tion by the method of Lineweaver and Burk [12]. Table 2 shows mean values obtained for both K_m and V_{max}. Both K_m and V_{max} values for the reduction of oestrone are considerably higher than for the oxidation of oestradiol. That such variation in the K_m and V_{max} value for $E_1 \rightarrow E_2$ is observed, presumably reflects the amount of endogenous enzyme regulators present in these crude enzyme preparations. In subsequent experiments where the initial velocity of reaction was measured, substrate concentrations as described in the incubation procedure were employed.

Experiments performed to consider cofactor requirements, have suggested that at the substrate concentration determined above, the conversion of oestrone to oestradiol was maximal at an NADH concentration of 1 mM. Enzyme activity was shown to be considerably lower with NADPH as cofactor. However, activity was enhanced to above that observed at saturating concentrations of NADH, when the cofactor generating system described under incubation procedure, was employed. In subsequent studies this cofactor generating system was used. The maximum rate of conversion of oestradiol to oestrone under conditions adopted to measure the initial velocity of reaction occurred in the presence of NAD at a concentration of 2 mM. Activity was shown to be negligible when NADP was used at a similar concentration.

Tissue concentration

A linear relationship between tissue concentration and product formed was demonstrated over the range 5–45 mg/ml for $E_1 \rightarrow E_2$ (Fig. 1a) and 5–55 mg/ml for $E_2 \rightarrow E_1$ (Fig. 1b) in both myometrium and leiomyoma tissue.

Incubation time and temperature

There was a linear relationship between the formation of oestradiol and time in both myometrium and leioyoma tissue for the duration of the experiment, i.e. 180 min (Fig. 2a). Similarly the formation of oestrone in both tissues used for this experiment was linear for 195 min (Fig. 2b). Enzyme activity in myometrium and leiomyoma ($E_1 \rightarrow E_2$, $E_2 \rightarrow E_1$) was low at 20°C rose to a maximum at around 40°C and fell to barely detectable levels at 65°C.

Interconversion of oestrone and oestradiol

The interconversion of oestrone and oestradiol in leiomyoma and normal myometrium with respect to the phase of the menstrual cycle is shown in Table 3. Conversion of oestradiol to oestrone was shown to be significantly higher in tumour tissue than in myometrium in the proliferative phase of the cycle

Table 3.

	$E_1 \rightarrow E_2$*		$E_2 \rightarrow E_1$*	
Phase of cycle	L	M	L	M
Early proliferative	237	476	121	89
(Days 5–8)	614	447	—	—
	916	1574	311	273
	580	336	719	365
Late proliferative	313	340	446	135
(Days 9–14)	414	463	446	336
	504	272	514	266
	635	969	712	504
	748	740	843	927
	413	394	265	141
Mean	537	601	486	337
SD	205	401	237	257
Early secretory	—	—	519	379
(Days 15–18)	199	160	—	—
	310	167	554	136
	1103	800	1467	563
	1514	1848	2786	2912
	4189	435	3029	303
Mid secretory	943	6559	147	1129
(Days 19–23)	721	939	932	1219
	634	1852	531	1585
Mean	1202	1595	1246	1028
SD	1279	2114	1097	915

*ng oestradiol or oestrone/g tissue/h.

17β-OHSD activity in leiomyoma (L) and myometrium (M) throughout the menstrual cycle. Tissues were grouped according to stage of cycle as determined by endometrial histology and day since the last menstrual period (where available). Conversion of oestradiol to oestrone was significantly higher ($P < 0.01$) in tumour than normal tissue in the proliferative phase.

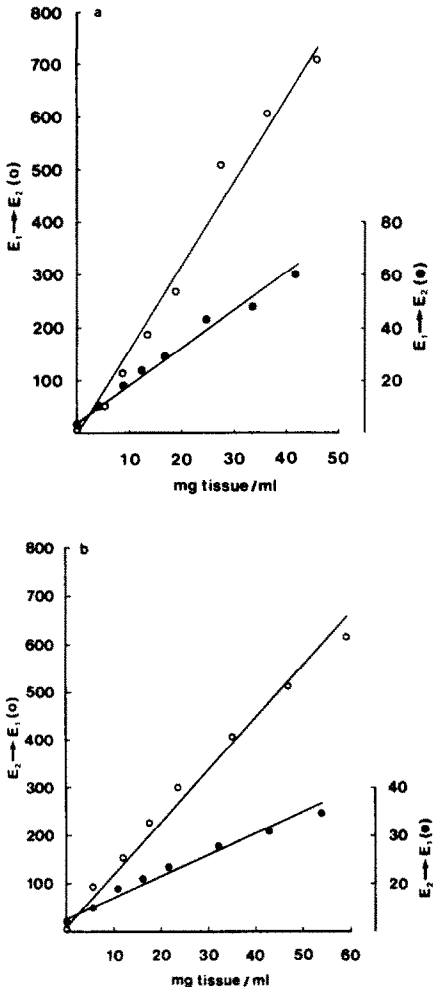

Fig. 1. The effect of homogenate concentration (g. wet wt tissue/ml buffer) on the conversion of a. oestrone to oestradiol ($E_1 \rightarrow E_2$) and b. oestradiol to oestrone ($E_2 \rightarrow E_1$) by 17 OHSD in both leiomyoma (○) and myometrium (●). Enzyme activity is expressed as pmol oestrone or oestradiol formed per hour.

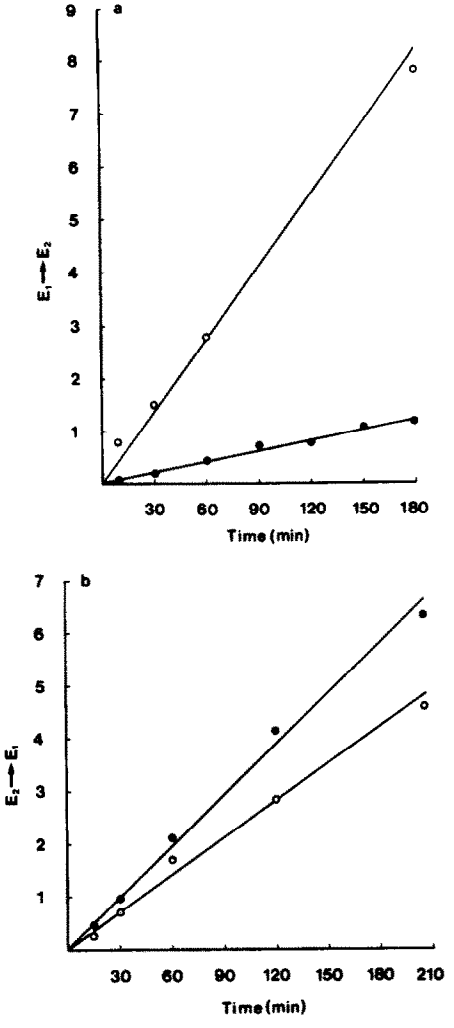

Fig. 2. Time course of the conversion of a. oestrone to oestradiol ($E_1 \rightarrow E_2$) and b. oestradiol to oestrone ($E_2 \rightarrow E_1$) by 17 OHSD in both leiomyoma (○) and myometrium (●). Enzyme activity is expressed as nmol oestrone or oestradiol formed per gram of tissue.

($P < 0.01$). No other significant difference in enzyme activity was noted between the two tissues. Table 3 indicates that in both tumour and normal tissue obtained during the secretory phase, large variations in activity occur between subjects. However, the mean values for the interconversion of oestrone and oestradiol ($E_1 \rightarrow E_2$, $E_2 \rightarrow E_1$) in both tissues were considerably higher in the secretory than the proliferative phase. Data from one secretory phase subject has been excluded from Table 3. Enzyme activity was

Table 4.

Tissue		$E_1 \rightarrow E_2$ (ng/g tissue/h)	$E_2 \rightarrow E_1$ (ng/g tissue/h)	Oestrone (pg/g tissue)	Oestradiol (pg/g tissue)	Progesterone (ng/g tissue)
Tumour A						
Section	i	20166	9119	1121	1364	11.9
	ii	22490	12688	1129	1037	9.7
	iii	25290	13072	1312	1100	9.7
	iv	10465	6286	1938	4012	12.4
Tumour B						
Section	i	277	482	1471	2667	9.9
	ii	223	482	1344	1454	7.8
	iii	225	528	1123	2017	7.0
	iv	246	639	1003	1288	8.6
Myometrium		1398	1785	1124	1692	7.9

Enzyme activity and tissue steroid concentrations measured in sections of two tumours and in myometrium from the same uterus.

Table 5.

Phase of cycle	Oestrone (pg/g tissue) L	Oestrone (pg/g tissue) M	Oestradiol (pg/g tissue) L	Oestradiol (pg/g tissue) M	Progesterone (ng/g tissue) L	Progesterone (ng/g tissue) M
Early proliferative	1802	1717	633	368	2.08	1.97
(Days 5–8)	405	554	459	732	1.17	0.97
	720	545	1920	1568	1.56	2.98
	771	803	384	343	2.91	4.16
Late proliferative	268	254	856	444	1.59	2.39
(Days 9–14)	2171	1608	1469	1395	1.97	2.69
	594	535	408	293	1.89	1.94
	2589	1272	1262	761	3.59	2.67
	1272	1576	2825	1983	3.18	4.68
	508	463	1716	853	1.00	0.91
Mean	1110	933	1193	874	2.09	2.54
SD	811	553	802	585	0.86	1.21
Early secretory	—	—	—	—	4.78	6.59
(Days 15–18)	375	390	512	320	3.68	4.58
	851	786	932	552	2.32	—
	759	770	853	638	4.29	7.88
	1150	829	1253	757	7.99	10.78
	695	733	772	857	3.16	6.32
Mid secretory	542	615	747	705	1.06	3.25
(Days 19–23)	767	590	692	559	3.48	4.87
	707	610	464	616	8.22	9.47
Mean	731	665	778	626	4.33	7.62
SD	226	143	249	161	2.40	2.55

Steroid concentrations in leiomyoma (L) and myometrium (M) throughout the menstrual cycle. As with enzyme activity, tissues were grouped according to endometrial histology and day since the last menstrual period. Proliferative phase concentrations of oestradiol were significantly higher in tumour than normal tissue ($P < 0.01$). Secretory phase progesterone concentrations were significantly higher in myometrial than tumour tissue ($P < 0.001$).

considered in sections of two tumours found in the uterus of this subject (Table 4). Activity in one tumour (A) was found to be very much higher than the values reported in Table 3. However, enzyme activity in tumour B and myometrium fell within the range of values shown in Table 3. It can also be observed that activity varies considerably across the section of tissue from tumour A.

Tissue concentrations of oestrone, oestradiol and progesterone

Tissue steroid concentrations measured in normal myometrium and leiomyoma are presented in Table 5. Oestradiol concentrations were shown to be significantly higher in tumour tissue than normal myometrium in the proliferative phase of the menstrual cycle ($P < 0.01$). Mean concentrations of oestrone and oestradiol were shown to be higher in both tissues in the proliferative than in the secretory phase, although considerable overlap in values between the two phases occurred. Progesterone concentrations were shown to be significantly higher ($P < 0.001$) in secretory phase myometrium than in corresponding tumour tissue. Concentrations in both tissues were considerably higher in the secretory than the proliferative phase of the menstrual cycle. Table 4 also shows steroid concentrations in two tumours corresponding to enzyme activity measurements previously described. Unlike enzyme activity, steroid concentrations are similar in both tumours, but variation was observed between sections taken from the same tumour.

Data from one postmenopausal subject who had been receiving steroid therapy for symptoms of the climacteric are presented in Table 6. Tissue enzyme activity and progesterone levels correspond to those observed in the proliferative phase of the menstrual cycle, but tissue oestrogens are considerably higher than those shown in Table 3. Of particular interest is the further observation that tissue oestrone and oestradiol concentrations are 6- and 3-fold higher respectively, in tumour tissue than normal myometrium. Consideration of this patient's history revealed that she had been taking steroid preparations for symptoms of the menopause since 1975. Over the 2 years prior to hysterectomy these preparations had contained oestrone sulphate, mestranol and norethisterone.

DISCUSSION

Evidence presented in this paper confirms that both normal myometrium and leiomyoma tissue con-

Table 6.

$E_1 \rightarrow E_2$ (ng/g tissue/h) L	$E_1 \rightarrow E_2$ (ng/g tissue/h) M	$E_2 \rightarrow E_1$ (ng/g tissue/h) L	$E_2 \rightarrow E_1$ (ng/g tissue/h) M	Oestrone (pg/g tissue) L	Oestrone (pg/g tissue) M	Oestradiol (pg/g tissue) L	Oestradiol (pg/g tissue) M	Progesterone (ng/g tissue) L	Progesterone (ng/g tissue) M
314	325	237	161	14935	2443	11481	3786	1.3	1.1

Enzyme activity and tissue steroid concentrations in leiomyoma (L) and myometrium (M) obtained from a postmenopausal subject receiving steroid therapy for symptoms of the climacteric.

tain 17β-OHSD, which can catalyse the interconversion of oestrone and oestradiol. From kinetic studies it appears that the favoured direction of operation of this reaction is towards the oxidation of oestradiol, as found by Tseng and Gurpide in endometrium [1]. These authors reported that the K_m was higher and the V_{max} was lower for the reduction of oestrone but in our study both K_m and V_{max} are higher. It is possible that where oestrone concentrations are high enough, as in the case of localised production of oestrone [8], substantial conversion to oestradiol might occur.

Another factor which may influence the direction of operation of this enzyme is cofactor concentration. The present study indicates that NAD, as opposed to NADP, is required for the conversion of oestradiol to oestrone. The situation with respect to the reverse direction is less clear. Even though activity was shown to be considerably higher with NADH as opposed to NADPH, the use of a system which produced the reduced forms of both cofactors, resulted in an increase in activity above that observed at saturating concentrations of NADH. The use of this system may be more appropriate, since it is likely that both NADH and NADPH will be present in the cell *in vivo*.

Our study does not support that of Pollow *et al.* [7] who reported that 17β-OHSD activity in leiomyoma tissue was lower than that observed in myometrium. Apart from the conversion of oestradiol to oestrone in the proliferative phase, where conversion in tumour tissue was shown to be greater than in myometrium, there was no significant difference in enzyme activity between tumour tissue and normal myometrium (Table 3). This observation is consistent with that of Gabb and Stone [13]. However, data presented in Table 4 indicate that considerable differences in 17β-OHSD activity can exist between individual tumours found in the same uterus.

The present study suggests that in both normal and tumour tissue, the 17β-OHSD activity and progesterone concentrations are higher in the secretory than the proliferative phase of the menstrual cycle. Progesterone has been shown to induce the synthesis of 17β-OHSD in endometrium [3]. The mechanism by which this is achieved probably involves the binding of progesterone to a cytosolic receptor protein, to form a complex. This permits the steroid to be carried into the nucleus where it can bind to chromatin and initiate protein synthesis. If this mechanism also operates in leiomyoma and myometrium, it is necessary to account for the finding that in some secretory phase tissue, enzyme activity is similar to that observed in the proliferative phase. One possible explanation is that an altered binding affinity or lack of receptor for progesterone may be responsible.

With the exception of oestradiol concentrations in the proliferative phase of the cycle, which will be considered later in this discussion, no significant difference in oestrone or oestradiol concentrations between tumour and normal tissue could be observed. However, tissue concentrations of oestrone and oestradiol in some subjects were shown to be higher in the proliferative than the secretory phase. Otubu *et al.* [14] have also shown that oestradiol concentrations in both tumour and normal tissue were higher in the proliferative than the secretory phase.

In view of the finding reported earlier in this paper that conversion of oestradiol to oestrone in both tissues is higher in the secretory than proliferative phase, one may expect to observe a lower oestradiol/oestrone ratio in the secretory compared to the proliferative phase of the cycle. When data from Table 5 are considered such a difference is not observed. Also there is no apparent increase in secretory phase concentrations of oestrone which one may have anticipated from the observed increase in enzyme activity mentioned above. However, *in vivo* studies performed by Wiegernick *et al.* [15] showed that infusion of labelled oestrone for 12 h failed to give rise in myometrium to a tissue/plasma ratio for oestrone greater than unity. This may imply that oestrone does not readily accumulate in myometrium and even if conversion of oestradiol is enhanced, this may not be reflected by increased tissue concentrations of oestrone. Until more is known of the details of tissue kinetics, it is difficult to interpret tissue concentrations in relation to metabolic activities.

One of the factors that is thought to determine tissue steroid concentrations is intracellular receptor site concentration. Several studies concerning the measurement of oestrogen and progesterone receptor site concentrations in leiomyoma and myometrium have been reported [16–21]. Estimates of oestrogen receptor site concentrations in three studies [19, 20, 21] have been shown to be higher in tumour than normal tissue. Our study and that of Otubu *et al.* [14], suggests that proliferative phase oestradiol concentrations are higher in leiomyoma than myometrium. Thus under conditions where plasma oestradiol is rising and conversion of oestradiol to oestrone is low (as in the proliferative phase), higher oestrogen receptor concentrations in tumour tissue may explain the higher oestradiol concentrations observed. The finding reported in the present study that progesterone concentrations in the secretory phase are higher in myometrium than in leiomyoma cannot be accounted for by proposing that myometrial progesterone receptor concentrations are higher than those of tumour tissue [18, 19]. A difference in progesterone concentrations between the two tissues in the secretory phase was not reported by Otubu *et al.* [14].

Coulson *et al.* [22] have shown that in the rat uterus oestradiol modulates the concentration of its own receptor. This has been shown to occur in two distinct phases. The first is a short term depletion of receptor. Continued exposure to oestradiol then leads to an

increase, to receptor concentrations above basal levels. The positive correlation observed between oestradiol and its receptor in the human uterus [6, 23], has led to the view that oestrogens induce their own receptor. It is possible that under a prolonged oestrogenic stimulus, leiomyoma tissue responds by producing more receptor than normal tissue. Data from a patient who had been receiving oestrogens prior to hysterectomy (Table 6) may provide evidence to support this hypothesis. Both oestradiol and oestrone levels are several fold higher in leiomyoma tissue than myometrium. The co-administration of the progestin norethisterone may have been expected to oppose the rise in oestrogen receptor concentration and to increase the activity of 17β-OHSD, as reported to occur in endometrium [24]. Clearly enzyme activity is not raised. Therefore the possibility exists, as suggested earlier in this report, that the progesterone receptor mechanism was impaired in tissues from this subject. Another explanation, is that these tissues may have been capable of converting norethisterone to ethinyl oestradiol, as reported to occur in human placental microsomes by Barberi *et al.* [25]. As norethisterone is a progestin used frequently in oral contraceptives and in preparations for symptoms of the menopause, the likelihood of this conversion occurring in myometrium and leiomyoma tissue should be investigated.

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