Journal of Steroid Biochemistry, 1975, Vol. 6, pp. 1489–1494. Pergamon Press. Printed in Great Britain

METABOLISM OF ESTRADIOL-17β IN HUMAN ENDOMETRIUM DURING THE MENSTRUAL CYCLE

BEVAN J. BUIRCHELL and ROLAND HÄHNEL

University of Western Australia, Department of Obstetrics and Gynaecology, King Edward Memorial Hospital for Women, Subiaco, 6008, Western Australia

(*Received* 26 *December* 1974)

SUMMARY

[^{3}H]-estradiol-17β was incubated with endometrium obtained at different times during the menstrual cycle. The percentage of the [^{3}H]-estradiol-17β metabolized was measured. Secretory endometrium metabolizes estradiol-17β at a greater rate than proliferative endometrium. Proliferative endometrium metabolizes estradiol-17β to estrone while secretory endometrium, under the influence of progesterone, metabolizes estradiol-17β to water soluble metabolites and a small quantity of estrone. The production and excretion of water soluble metabolites by the secretory endometrium is rapid. Endometrium showing cystic hyperplasia or adenocarcinoma metabolizes estradiol-17β at the same rate as proliferative endometrium.

INTRODUCTION

Metabolic transformation of estradiol-17β has been consistently demonstrated in human endometrium [1–4] and myometrium [1]. The major metabolite isolated was estrone. Ball and Knuppen [5] also isolated estrone along with quantities of 6α-hydroxy-estradiol-17β and two water soluble metabolites, estradiol-17β sulphate and a positively charged conjugate.

Several workers have investigated the cyclic pattern of uptake and metabolism of estradiol-17β in superfused tissue [3, 6], homogenates [7], and tissue slices [1, 4]. Tseng and Gurpide[3] revealed differences in the rate of conversion of estradiol-17β to estrone in the proliferative and secretory phase and they interpreted this as being due to an alteration in estradiol-17β dehydrogenase activity. In a later investigation Tseng and Gurpide[7] measured the activity of estradiol-17β dehydrogenase in 800*g* supernatant and found a thirteen-fold increase in the enzyme activity following ovulation. The current investigation, carried out in tissue slices, also demonstrates this increase in estradiol-17β dehydrogenase activity but shows that water soluble metabolites, not estrone, are the major metabolites during the secretory phase.

MATERIALS AND METHODS

Chemicals. Reagent grade organic solvents were redistilled before use. [6,7-^{3}H]-estradiol-17β (S.A. 45 Ci/mmol) was purchased from Radiochemical Centre, Amersham, England. Unlabelled steroids and Sulphatase (Aryl Sulphatase) Type III: from Limpets were obtained from Sigma Chemical Co., St. Louis, Missouri, U.S.A.

Tissues. The human endometrium used in this study was obtained from uteri removed by hysterectomy. Decidual tissue was obtained at therapeutic abortion.

Method

Uteri obtained at hysterectomy were immediately placed on ice. The endometrium was removed and a portion used for histological identification. The remainder was sliced into pieces weighing 20–50 mg wet weight. Tissue incubations were generally conducted as follows: 2·8–3·1 pmol of [6,7-^{3}H]-estradiol-17β (S.A. 45 Ci/mmol) was incubated at 37°C with 100–150 mg of endometrium for 1 h in 5 ml of phosphate buffer of the following composition: 0·05 M Sodium–potassium phosphate (pH 7·4), 0·025 M KCl and 0·004 M $MgCl_2$ [1]. At the end of the time period the tissue slices were removed and placed into another flask containing 5 ml of phosphate buffer.

This flask, together with that containing the original incubation medium were flooded with 16 ml acetone and left overnight at 4° C. Throughout the publication these extracts will be referred to as the "tissue fraction" and "medium fraction" respectively.

The extracts were filtered, to remove precipitated proteins and tissue, and the acetone evaporated under N_2. The remaining aqueous phase was extracted with ether (3 × 15 ml) and the combined etheral extracts were dried over Na_2SO_4. After evaporation, the ether extract afforded a residue which was dissolved in ethanol (2·5 ml).

Investigation of the ether phase components on Silica Gel G (chloroform-ethylacetate 9:1 v/v) revealed that radioactivity was localized at positions corresponding to standard estradiol-17β and estrone. These active zones were scraped into counting vials and Permablend-toluene scintillator (10 ml) added. Radioactivity was then measured in a Tri-carb model 3375 liquid scintillation spectrometer with automatic external standardization. From this the amount of estrone was calculated as a percentage of the original [^{3}H]-estradiol-17β added. Column chromatography

of the residue on Sephadex LH-20, using dichloromethane-methanol (85:15 v/v) as elution solvent also indicated estrone to be a metabolite. This was confirmed by recrystallization, from hexane–acetone, to constant S.A.

The radioactivity in the aqueous phase was measured in Bray's scintillation mixture [8]. The aqueous phase contains the water soluble metabolites. The water soluble metabolites were applied to a Florisil (60–100 mesh) column prepared according to the directions of Collins[15]. The column was eluted with 50 ml fractions each of ethylacetate, ethylacetate–methanol (4 : 1, 1 : 1, 1 : 4 by vol.) methanol and methanol:water (4:1, 1:1, 1:4 by vol.). Eluates of 50 ml were collected, evaporated to dryness and reconstituted in 2 ml methanol. Aliquots of the eluates were counted in order to determine which contained the radioactive metabolites.

The ^3H estrogen sulphates which eluted in the second and third fractions were chromatographed on Celite 545 using the solvent system chloroform–n-heptane t-butanol 1·0 N NH_4OH (1:2:2:2 by vol.). The Celite column was prepared according to the directions of Preedy and Aitken[16].

Thin-layer chromatography, of the water soluble metabolites was carried out on Silica Gel G in two separate solvent systems:— (1) methylene chloride: methanol:ammonia (70:22:8 by vol.) [17]. (2) n-butanol after equilibration for two hours in 10% NH_4OH in water [18]. Appropriate standards of estrone sulphate and estradiol-3-sulphate were run with each plate.

The tritiated metabolites isolated from the Florisil column, Celite column and thin-layer chromatography plates were treated with Sulphatase (24 h at 37°C) and extracted with ether. The resulting free estrogens were identified by thin layer chromatography in chloroform–ethylacetate (9:1 v/v). Estrone and estradiol were recrystallized to constant specific activity from hexane–acetone.

RESULTS

There are two types of metabolites produced by human endometrium from estradiol-17β, free (ether-extractable) and conjugated (water soluble) metabolites. Estrone was isolated and identified as the free metabolite of estradiol-17β by t.l.c., column chromatography on Sephadex LH-20 and by recrystallization to constant S.A.

Florisil elution chromatography was used to differentiate the water soluble metabolites into sulphates and/or glucosiduronates. Characteristically, estrogen sulphates are eluted with ethylacetate–methanol (4:1, 1:1, by vol.) where as estrogen glucosiduronates are eluted by methanol and methanol–water (4:1, v/v) [15]. The water soluble metabolites produced by secretory endometrium elute in the ethyl acetate–methanol (4:1, 1:1, by vol.) fractions.

Separation of the sulphate fraction from Florisil column was achieved by chromatography on Celite 545 (Fig. 1). Estrone sulphate which contains 80% of the water soluble ^3H is recovered in the 4–7 ml eluate. Estradiol sulphate which contains the rest of the water soluble ^3H is recovered in the 10–15 ml eluate.

Identification of the estrogen sulphates was also achieved by thin-layer chromatography. Figure 2 is a typical chromatogram of the water soluble metabolites run in methylene chloride methanol ammonia (70:22:8, by vol.). The water soluble ^3H metabolites have the same mobility as the authentic standards of estrone sulphate (R_F = 0·48) and 17β-estradiol-3-sulphate (R_F = 0·35). The majority of the radioactivity is associated with the estrone sulphate standard. Similar separation and identification of the water soluble metabolites was achieved in the second solvent system. (R_F Estrone sulphate = 0·70, 17β-estradiol-3-sulphate = 0·37).

Treatment of the individual sulphate peaks with Sulphatase, subsequent extraction and thin-layer chromatography, identified the free estrogens corresponding to their respective sulphates.

Quantitative and qualitative variations in metabolites occur with changes in the menstrual cycle. Figure 3 shows the percentage conversion of [^3H]-estradiol-17β into estrone and water soluble metabolites during the menstrual cycle. In the mid-proliferative phase 26·9 ± 2·7% (mean ± standard error of the mean) of the [^3H]-estradiol-17β is metabolized, the majority to estrone. In the later proliferative phase the conversion drops to 20·4 ± 1·7% with estrone again being the major metabolite.

Just before or after ovulation there is a sudden increase in the quantity and an alteration in the nature of the metabolites produced. In one experiment there was a rapid increase in estrone levels (36%) above that of the proliferative phase and an increase in water soluble metabolites (22·6%). Prior to the early secretory phase the major conversion of [^3H]-estradiol-17β changes to water soluble metabolites instead of estrone (Fig. 3). Mid secretory endometrium has

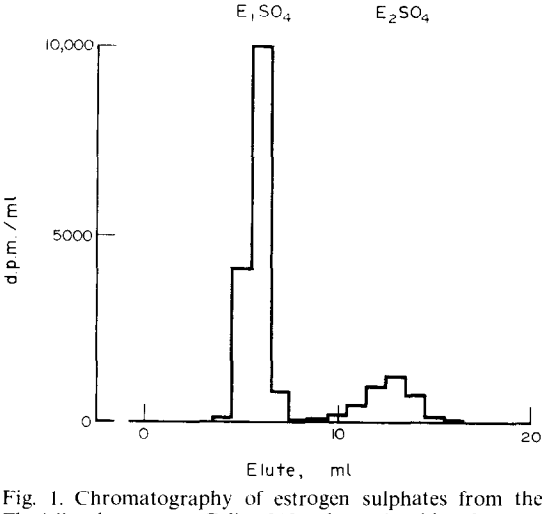

Fig. 1. Chromatography of estrogen sulphates from the Florisil column on a Celite 545 column in chloroform–n-heptane–tertiary butanol–1 M NH_4OH (1:2:2:2 by vol.)

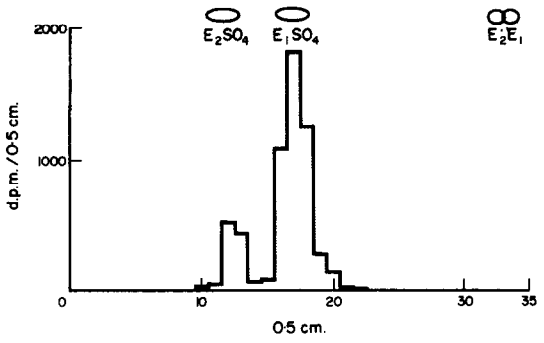

Fig. 2. Thin-layer chromatography of the sulphate fraction from the Florisil column. The standard E_2-3-SO_4 and E_1-3-SO_4 had R_F values of 0·35 and 0·48 respectively (circles). Solvent system methylene chloride–methanol–ammonia (70:22:8 by vol.).

the greatest metabolic activity converting 63·3 ± 3·5% of the [^{3}H]-estradiol-17β to water soluble metabolites and 17·1 ± 1·7% to estrone. Towards the end of the cycle the level of water soluble metabolites decreases (26·1 ± 4·7%) while estrone, again becoming the dominant metabolite, is increased in production to 33·7 ± 3·8%.

Decidual endometrium, obtained during the first trimester, converts 47·1 ± 4·1% of the [^{3}H]-estradiol-17β to estrone which is slightly below the conversion to total metabolites at the end of the cycle (59·8 ± 4·6%, Fig. 3). The level of water soluble metabolites (5·4 ± 0·4%) is higher than in the proliferative phase (3·1%) but is considerably lower than that produced in the secretory phase (63·3%).

Myometrium, in contrast to endometrium, does not have a cyclic dependent metabolic pattern and the percentage conversion is below that of endometrium in all phases of the cycle. Myometrium metabolizes 6·6 ± 1·2% of the [^{3}H]-estradiol-17β to estrone and 3·8 ± 0·6% to water soluble metabolites.

Figure 4 shows the results of a time course experiment in which [^{3}H]-estradiol-17β was incubated with a secretory endometrium and the level of estrone and water soluble metabolites was measured at 30 min intervals. In the first 30 min 54·3% of the [^{3}H]-estradiol-17β has been metabolized, mainly to water soluble metabolites (34·6%) and the estrone level is only 19·7%. The percentage of total [^{3}H]-estradiol-17β metabolism continues to increase to 87·9% at 90 min then it flattens off. The continuing rise in total metabolite level is predominantly due to the formation of water soluble metabolites since estrone levels never rise above the initial value of 19·7% (Fig. 4).

More detailed information regarding the distribution of metabolites in the incubation medium and in the tissue with time is given in Table 1. At 30 min the level of total metabolites in the medium (37·8%) is more than twice that in the tissue (16·5%). The total amount of metabolites in the tissue remains constant after 30 min. The percentage of estrone in the medium remains almost constant after the first 30 min, but the content of water soluble metabolites increases rapidly to 63·4% at 90 min then flattens off. It can be seen from these two sets of results that the secretory endometrium is geared towards rapid production and excretion of water soluble metabolites.

In comparison Fig. 5 and Table 2 demonstrate the same process in a late proliferative tissue where water soluble metabolites are of little significance. While water soluble metabolites remain at a low level throughout the incubation, estrone increases linearly with time. Estrone concentration in the tissue and medium increases at a constant rate over the time course. The level of estrone in the medium increases at a slightly higher rate than in the tissue and the T/M (% Total metabolism in tissue/% Total metabolism in medium) ratio only varies 0·09 from 0·59 at 30 min to 0·50 at 120 min (Table 2). In contrast the T/M ratio in a secretory endometrium varies by 0·26 from 0·44 at 30 min to 0·18 at 120 min (Table 1).

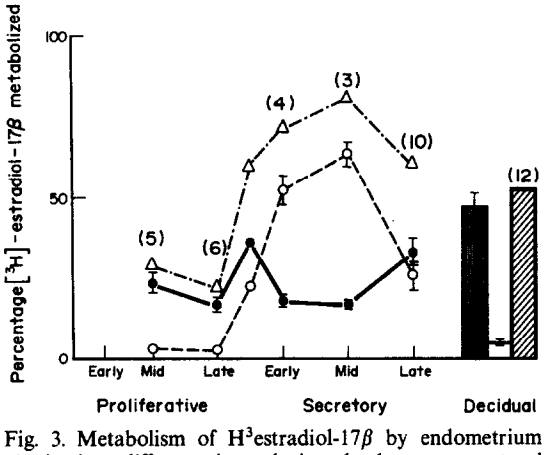

Fig. 3. Metabolism of H^{3}estradiol-17β by endometrium obtained at different times during the human menstrual cycle. The vertical bars show the standard error of the mean. ●——● ■ Estrone; ○----○ □ Watersoluble metabolites: △- · · △ ▨ Total metabolites. Numbers of experiments are given in parentheses.

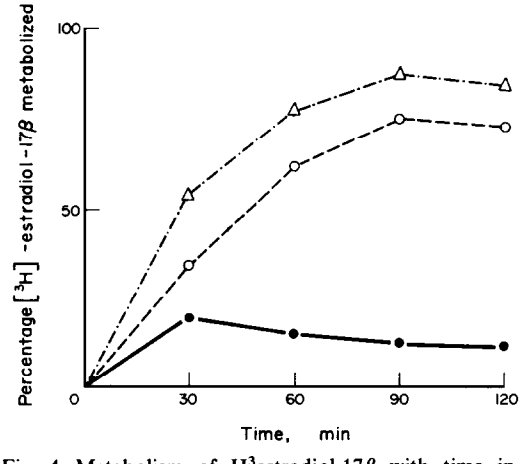

Fig. 4. Metabolism of H^{3}estradiol-17β with time in a secretory endometrium. ●——● Estrone; ○----○ Watersoluble metabolites; △—·—△ Total metabolites.

Table 1. Distribution of metabolites of estradiol-17β from a secretory endometrium. Figures expressed as the percentage of [^{3}H]-estradiol-17β metabolized. E_1 = Estrone; WS = Watersoluble metabolites

TIME (minutes)	MEDIUM			TISSUE			Tissue/ Medium
	E_1	WS	Total	E_1	WS	Total	
30	15.4	22.4	37.8	4.3	12.2	16.5	0.44
60	12.8	46.0	58.8	2.3	16.1	18.4	0.31
90	9.7	63.4	73.1	2.7	12.0	14.7	0.20
120	9.5	62.8	72.3	2.5	10.3	12.8	0.18

Table 2. Distribution of metabolites of estradiol-17β from a proliferative endometrium. Figures expressed as the percentage of [^{3}H]-estradiol-17β metabolized. E_1 = Estrone; WS = Watersoluble metabolites

TIME (minutes)	MEDIUM			TISSUE			Tissue/ Medium
	E_1	WS	Total	E_1	WS	Total	
30	2.7	1.1	3.8	1.9	0.3	2.2	0.59
60	4.6	1.3	5.9	3.1	0.2	3.3	0.56
90	9.8	1.7	11.5	5.7	0.2	5.9	0.51
120	11.9	2.0	13.9	6.6	0.3	6.9	0.50

Various pathological and endocrine disorders have been investigated to give a further insight into the factors controlling estradiol-17β metabolism. Table 3 demonstrates the results in patients with endometrial adenocarcinoma, endometrial cystic hyperplasia and anovulatory hyperplasia. There are four adenocarcinomatous tissues having poorly to well differentiated tumours. In all cases the amount of estrone produced (19·0%-28·9%) falls within the range for proliferative endometrium. The production of water soluble metabolites was negligible except in one case. Case Kin (28·9% water soluble metabolites) had been treated with 200 mg/day of "Provera" or medroxy progesterone acetate, a potent progestational agent.

Endometrial cystic hyperplasia and endometrium from patients with anovulatory hyperplasia metabolize estradiol-17β at the same rate as proliferative tissue.

DISCUSSION

The metabolism of estradiol-17β in human endometrium has a biphasic pattern which parallels the hormonal and morphological changes that occur in the menstrual cycle. In the proliferative phase estradiol-17β is oxidized to estrone by estradiol-17β dehydrogenase. The metabolic fate of estradiol-17β, in the secretory phase is different from that in the proliferative phase. Although estradiol-17β is still oxidized to estrone, conjugating enzymes further metabolize estradiol-17β and estrone to form water soluble metabolites. Seventy to eighty five per cent of the water soluble metabolites are derived from estrone, that is oxidation should occur before conjugation. Tseng and Gurpide[7] measured the activity of estradiol-17β dehydrogenase in 800 *g* supernatants during the menstrual cycle and the enzymic pattern was similar to the pattern observed when total metabolites were measured (Fig. 3).

The increase in metabolism and the appearance of water soluble metabolites correspond to the increase in progesterone secretion. The effect of progesterone on endometrium cannot be detected, histochemically, for at least 36–48 h after its secretion [9]. However, progesterone can be detected before this by the changes it induces in estradiol-17β metabolism. Initially there is an increase in both estradiol-17β dehydrogenase and conjugating enzymes and the metabolic pattern shows an increase in both estrone and water soluble metabolites. After a certain period of time the increase in conjugating enzymes is sufficient to metabolize the majority of estrone produced. Consequently there is a crossover point when water soluble metabolites take over, from estrone, as the major metabolites.

At the end of the menstrual cycle the activity of estradiol-17β dehydrogenase remains constant while the activity of the conjugating enzymes decreases. The

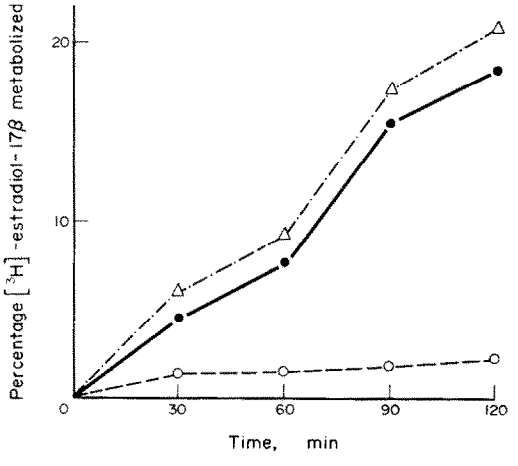

Fig. 5. Metabolism of H^{3}estradiol-17β with time in a proliferative endometrium. ●——● Estrone; ○——○ Watersoluble metabolites; △···—△ Total metabolites.

Table 3. Estradiol-17β metabolism by endometrium with adenocarninoma, cystic hyperplasia or anovulatory hyperplasia. Figures expressed as percentage of [^{3}H]-estradiol metabolized

DISORDER	STAGE	ESTRONE	WATERSOLUBLE	TOTAL
Adenocarcinoma:				
UND.	quite poorly differentiated	10,0	2,2	12,2
CLI.	moderately well differentiated	22,4	3,6	26,0
LAT.	well differentiated	20,3	2,9	23,2
KIN. *	moderately well differentiated	28,9	28,9	57,8
Hyperplasia:				
CON.	Cystic	27,9	2,3	30,2
WAT.	Cystic	5,9	2,2	8,1
GIB.	Cystic	12,5	2,2	14,7
BAI.	Cystic	27,0	2,8	29,8
MAR.	Anovulatory	13,2	2,0	15,2
BUR.	Anovulatory	18,8	2,6	21,4

* Treated with 200 mg/day with medroxyprogesterone acetate.

metabolic pattern is again altered and estrone becomes the major metabolite (Fig. 3). The high activity of estradiol-17β dehydrogenase and the presence of conjugating enzymes are not observed in the next menstrual cycle because all the endometrium, except the basalis layer, is expelled during menstruation [9]. The enzyme levels in the next cycle are those which exist in all non-progesterone treated endometria.

If conception occurs, progesterone and estradiol-17β concentrations increase above normal instead of decreasing at the end of the cycle. It is interesting to note that the water soluble metabolites in the decidual endometrium are very low, while estradiol-17β dehydrogenase activity is the same as it was in the late secretory phase. This may indicate that the conjugating enzymes are a "once-a-cycle" phenomenon, that is progesterone induces the production of a certain quantity of enzymes which only remains active till the end of the cycle.

Estradiol-17β is the primary growth-stimulating factor for endometrium [13]. The endometrium has a two phase cycle:— (1) The proliferative phase or the growing period where plasma estradiol-17β concentration increases, reaching a maximum before ovulation and (2) The secretory phase—the secreting or functional period where plasma estradiol-17β concentration is half the peak concentration but still relatively high. For a tissue to have a non-growing period when the growth factor is in high concentration, it must have the capacity to regulate, either the concentration of estradiol-17β directly, or the influence which estradiol-17β has on the genetic material. A combination of these two systems seems to occur to limit the influence of estradiol-17β on the endometrium. Tseng and Gurpide[7] have also suggested that the function of estradiol-17β metabolism is to limit the duration of estrogen action on the human uterus. A similar suggestion has been made by Jellinck and Lyttle[10] working with the rat uterus.

In the proliferative phase the endometrium has only a slight control over intracellular estradiol-17β levels since estradiol-17β is oxidized to estrone by low levels of estradiol-17β dehydrogenase. The remaining unmetabolized estradiol-17β is available to a high concentration of estradiol-17β receptors, for growth processes [14]. Consequently the inability of proliferative tissue to metabolize estradiol-17β is reflected in the abundant growth of the tissue.

Tseng and Gurpide[7] showed that estradiol-17β dehydrogenase and estradiol-17β receptor concentration change in opposite directions during the menstrual cycle. In the secretory phase metabolism is at its greatest while receptor levels are tending towards a minimum [14]. The small quantity of unmetabolized estradiol-17β has only a low concentration of receptors to bind. This limits the effect of estradiol-17βon the genetic material in the secretory phase. Therefore the concentration of estradiol-17β available to the tissue is only sufficient to maintain the cells and not for growth.

The water soluble metabolites produced in the secretory phase have properties which make them ideal products for limiting the action of estradiol-17β, since they are produced and excreted rapidly (Fig, 4, Table1). As sulphates of estrone and estradiol-17β they possess a low affinity for the estradiol-17β receptor and would only be bound if present at high concentrations [11]. The concentration of water soluble metabolites in secretory tissue is kept at a minimum due to their favourable excretion rate. So it can be seen from rates of metabolism and receptor concentrations that secretory tissue in contrast to proliferative tissue has a greater influence over limiting intracellular estradiol-17β concentrations and thus growth.

There are several pathological disorders where the inability of the tissue to control intracellular estradiol-17β levels results in abnormal growth. Excessive estrogen production or persistent stimulation by low levels of unopposed (by progesterone) estradiol-17β is regarded as a causative factor or cofactor for endometrial cystic hyperplasia and endometrial carcinoma [12]. In both these disorders metabolism of estradiol-17β occurs at the same rate as in proliferative tissue. In these cases progesterone is not present to stimulate estradiol-17β dehydrogenase and the conjugating enzymes and thus limit estradiol-17β influence over growth. Both pathological disorders can be treated with progestational agents [12]. Hormones, such as medroxyprogesterone acetate and 17α-hydroxyprogesterone caproate, are thought to act by limiting the synthesis of nucleic acids and altering the intra-cellular metabolism or by depressing other potentially tumour-stimulating compounds [12]. It is suggested here that they act by increasing the metabolism of estradiol-17β which limits the effect of the hormone on nucleic acid synthesis.

REFERENCES

1. Sweat M. L., Bryson M. J. and Young R. B.: *Endocrinology* **81** (1967) 167–172.
2. Sweat M. L. and Young R. B.: *Biochim biophys. Acta* **296** (1973) 189–195.
3. Tseng L. and Gurpide E.: *Am. J. Obstet. Gynec.* **114** (1972) 1002–1008.
4. Collins W. P., Mansfield M. D., Bridges E. C. and Sommerville I. F.: *Biochem. J.* **113** (1969) 399–407.
5. Ball P. and Knuppen R.: *Acta Endocr., Copenh.* Suppl. **173** (1973) 3.
6. Tseng L., Stolee A. and Gurpide E.: *Endocrinology* **90** (1972) 390–404.
7. Tseng L. and Gurpide E.: *Endocrinology* **94** (1974) 419–423.
8. Bray G. A.: *Anal. Biochem.* **1** (1960) 279.
9. *Histopathology of Endometrium.* (Edited by G. Dallenbach-Hellweg). Spring-Verlag, Heidelberg, New York, 1971. p. 65.
10. Jellinck P. H. and Lyttle C. R.: In *Advances in Enzyme Regulation* (Edited by George Weber). Pergamon Press, New York, Vol. 11 (1972) pp. 17–33.
11. Hähnel R., Twaddle E. and Ratajczak T.: *J. steroid Biochem,* **4** (1973) 21–31.
12. *Endocrine Therapy in Malignant Disease* (Edited by B. A. Stoll). W. B. Saunders Co. Ltd. London, 1972 p. 326.

13. *The Uterus* (Edited by H. J. Norris, A. T. Hertig and M. R. Abell). The Williams and Wilkins Company, Baltimore 1733, p. 227.
14. Evans L. H., Martin J. D. and Hähnel R.: *J. clin. Endocr. Metab.* **38** (1974) 23–32.
15. Collins D. C.: *Steroids* **18** (1971) 341–358.
16. Preedy J. R. K. and Aitken E. H.: *J. biol. Chem.* **236** (1961) 1300–1311.
17. Gabb R. G. and Stone G. M.: *J. Endocr.* **62** (1974) 109–123.
18. Pack B. A. and Brooks S. C.: *Endocrinology* **95** (1974) 1680–1690.

ADDENDUM

After the submission of this paper for publication, Gabb and Stone[17] published similar results for the cyclic metabolism of estradiol in the human uterus. Pack and Brook[18] also found cyclic metabolism of estradiol in the gilt uterus.