Culture of epithelial and stromal cells of guinea-pig endometrium and the effect of oestradiol-17 β on the epithelial cells

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Summary. Epithelial and stromal cells of guinea-pig endometrium were separated by enzymic digestion, isolated by successive centrifugation, and maintained in culture as pure cell types for 5 days on growth medium. On Day 5, ultrastructural studies were performed on the two cell types, demonstrating that epithelial cells can grow as a monolayer composed of cohesive groups of polygonal cells (1.3×10^5 cells/cm²), while stromal cells were mostly fibroblastic. The effect of hormones was studied on the epithelial cells in culture. The monolayer was cultured into harvest medium for 3 days to ensure the complete removal of endogenous steroids, then these cells were incubated with 2×10^{-9} M-oestradiol-17 β for 3 days. There was a rise in the progesterone receptor level, varying from 1.3 to 10.8 times. The three enzymes known to interfere with oestradiol-17 β metabolism were present in the epithelial cells grown in our culture conditions. By incubation with oestrone sulphate for 3 days it was demonstrated that, in cultured epithelial cells, (1) oestrone sulphate is converted into oestradiol-17 β sulphate, and (2) oestrogen sulphates are hydrolysed to active oestrogens.

Introduction

In-vitro culture methods offer a powerful tool for direct studies of hormonal actions on endometrium in well defined conditions that otherwise could not be obtained by in-vivo experimentation. Two kinds of in-vitro systems have been developed for the study of steroid action on endometrial tissues.

Uterine explants in organ culture have been used, preserving uterine morphology and histological differentiation. Kaufman *et al.* (1980) have described an organ culture method for human endometrium, Shapiro, Dyer & Colas (1980) have used an organ culture system to evaluate the response of human proliferative endometrium to progesterone stimulation, Clarke, Adams & Wren (1982) have established that progesterone induces 17 β -hydroxysteroid dehydrogenase in this human culture system, and Chaminadas, Prost, Propper, Agnani & Adessi (1982) have measured the steroid sulphatase activities in human endometrium grown in organ culture. Adler, Alberghini, Counts & Auletta (1983) have studied the secretion of mucin by explants of rabbits and human cervix in organ culture, and Sumida, Gelly & Pasqualini (1983) have established an organ culture system for fetal uterus of guinea-pig to study the control of progesterone receptor induction. Cell cultures appear preferable when the aim is to determine how hormones regulate the cellular differentiation of the endometrium. Eckert & Katzenellenbogen (1981) have grown a primary culture of human endometrium containing both epithelial and stromal cells which are hormone-responsive; other authors have used human endometrium cell cultures after separation of two cellular types for hormonal and metabolic studies (Satyaswaroop, Fleming, Bressler & Gurpide,

1978; Fleming & Gurpide, 1981; Korte, MacDonald, Johnston, Janice & Casey, 1983; Tseng, 1984). Rajkumar, Bigsby, Lieberman & Gerschenson (1983) have obtained uteroglobin production by rabbit epithelial cell culture, but the health and cellular type of the cells was not checked ultra-structurally. Morphological characteristics and hormonal studies have been carried out together by Bell & Searle (1981) who studied the differentiation of decidual cells in mouse endometrium, by Ricketts, Hagensee & Bullock (1983) investigating uteroglobin production by the two cellular types of rabbit endometrium in culture, by Echeverria, Vazquez-nin & Pedron (1980) studying the response of rat endometrium to oestradiol-17 β in culture, by Siegfried, Nelson, Martin & Kaufman (1984) for histochemical identification of cultured cells from human endometrium and by McCormack & Glasser (1980) who cultured separately epithelial, stromal and myometrial cells of rat uterus and studied the response of each cellular type to oestradiol-17 β *in vitro*. Until now such studies have not been reported on guinea-pig endometrium.

The effects of oestrogen on guinea-pig endometrium have been reported by Sumida *et al.* (1983) and Lanzone, Nguyen & Pasqualini (1983) who described an hormonal control of the progesterone receptor synthesis in the fetal uterus. Laure & Pasqualini (1983) have demonstrated that oestradiol-17 β increased the RNA polymerase in fetal uterus. Moutaouakkil, Prost, Dahan & Adessi (1984) have studied sulphatase activities in the uterus, and a metabolic pathway of oestrogens in pregnant female guinea-pigs has been proposed (Adessi, Tran Quang Nhuan & Prost, 1981; Adessi, Tran Quang Nhuan & Vingler, 1982).

In the present study, we have developed epithelial and stromal cell primary cultures of guinea-pig endometrium, and characterized their ultrastructural features in transmission and scanning electron microscopy. Then, the culture conditions for the study of oestrogen action on progesterone receptor induction and oestradiol-17 β and oestrone sulphate metabolism in this epithelial cell culture system were determined.

Materials and Methods

Chemicals and reagents. CMRL 1066 medium, Hank's Balanced Salt Solution (HBSS), Ca²⁺- and Mg²⁺-free HBSS (CMF-HBSS), penicillin, streptomycin, gentamycin, fungizone, Hepes buffer, fetal calf serum (FCS) and horse serum were obtained from Flow Laboratories (Puteaux, France). A steroid-free serum substitute (Ultrosor SF) was obtained from IBF (Villeneuve la Garenne, France). Oestradiol-17 β , oestrone-3-sulphate, dehydroepiandrosterone-3-sulphate (DHEA sulphate), diethylstilboestrol, cortisol, dithiothreitol and ethylenediamine tetracetic acid (EDTA) were purchased from Sigma Chemical Co. (St Louis, MO, U.S.A.). [6,7-³H]Oestrone sulphate (ammonium salt) (sp. act. 53.0 Ci/mmol), [7-³H]DHEA sulphate (ammonium salt) (sp. act. 24.0 Ci/mmol), [4-¹⁴C]DHEA (sp. act. 57.8 mCi/mmol), [methyl-3-³H]R 5020 (sp. act. 80 Ci/mmol) and unlabelled R 5020 were obtained from New England Corporation (Boston, MA, U.S.A.). [2,4,6,7-³H]Oestradiol-17 β (sp. act. 100.0 Ci/mmol) was purchased from the Commissariat à l'Energie Atomique (Gif sur Yvette, France). [4-¹⁴C]Oestrone (sp. act. 52.0 mCi/mmol) was obtained from the Radiochemical Centre (Amersham, U.K.).

Preparation of guinea-pig endometrial cell cultures and culture conditions. Guinea-pigs, weighing 400–500 g, of the Hartley albino variety, were purchased from the Centre de Zootechnie Coblanbel (Montmédy, France). The animals were maintained under controlled temperature (20°C) and lighting conditions (12 h light:12 h dark). They were fed *ad libitum* with standard food enriched with ascorbic acid. Uteri were removed from mature virgin females killed on the day of vaginal opening, and placed in sterile HBSS containing penicillin (1000 U/ml), streptomycin (1000 µg/ml), and 10 mM-gentamycin buffered to pH 7.4 with 20 mM-Hepes. All procedures were conducted under sterile biological conditions. The myometrium was torn off, then the endometrium was minced into 2.5 mm cubes and washed three times in sterile CMF-HBSS containing streptomycin (100 µg/ml), penicillin (100 U/ml) and buffered to pH 7.4 with 20 mM-Hepes.

Cells were dissociated according to the method of Satyaswaroop, Bressler, de la Pena & Gurpide (1979) as modified by Varma *et al.* (1982). The endometrial fragments were incubated in 0.25% (w/v) collagenase, dissolved in CMF-HBSS buffered to pH 7.4 with 20 mM-Hepes, for 2 h at 37°C. The cell dissociation was monitored by phase-contrast microscopy. After dissociation, the undigested fragments were collected by sedimentation. The glands and the supernatant cell suspension were centrifuged at 100 g for 5 min. The pellet containing epithelial glands was washed three times in 5 ml CMF-HBSS then centrifuged at 100 g for 5 min. The remaining pellet containing approximately 95% of glandular epithelial cells was plated in the growth culture medium (see below), 10^3 epithelial cell clusters per ml (in 25 cm² tissue culture flasks; Falcon). After gland isolation, the supernatant was centrifuged at 400 g for 10 min, then the pellet containing the stromal cells was plated in growth culture medium (10^5 cells/ml).

The growth culture medium was CMRL 1066 supplemented with 10% FCS, penicillin (100 U/ml), streptomycin (100 µg/ml), fungizone (2.5 µg/ml), 10 mM-L-glutamine, glucose (5 mg/ml), insulin (5 µg/ml), NaHCO₃ (0.85 mg/ml), 20 mM-Hepes buffer and 10^{-9} M-oestradiol-17β. The flasks were incubated in a humidified atmosphere composed of 5% CO₂ and 95% air.

The plated cultures reached confluence within 5–6 days. The medium was changed every 3 days. For experiments, after 5–6 days the growth medium was replaced by fresh harvest medium: CMRL 1066, penicillin (100 U/ml), streptomycin (100 µg/ml), fungizone (2.5 µg/ml), 10 mM-L-glutamine, glucose (5 mg/ml), insulin (5 µg/ml), NaHCO₃ (0.85 mg/ml), 20 mM-Hepes and 10% FCS pretreated with dextran-charcoal, 2.5% horse serum or 2% Ultrosor SF.

To ensure the complete removal of endogenous steroids, the harvest medium was changed every day for 3 days, and the concentrations of oestradiol-17β, oestrone and oestrone sulphate in the medium were measured each day.

To stimulate progesterone receptor synthesis, the cells were cultured 3 more days in fresh harvest medium supplemented with 2×10^{-9} M-oestradiol-17β, then harvested and processed for biochemical studies. Oestrone sulphate metabolism was studied in epithelial cells cultured during 3 days in harvest medium containing 10^{-7} M-oestrone sulphate renewed each day. On Day 3, unconjugated and conjugated oestrogens were identified by mass-spectrometry in the harvest culture medium.

The growth of the monolayers was followed daily with an Olympus T01 inverted phase-contrast microscope. For examination by scanning electron microscopy, the cell cultures were carefully rinsed and fixed in 0.1 M-sodium cacodylate-buffered 2% glutaraldehyde, pH 7.4, for 30 min. After dehydration with acetone, the specimens were transferred into liquid CO₂ for critical-point drying. Sputter-coated pieces of cell culture were viewed in an Autoscan Etec scanning electron microscope (Siemens, St Denis, France).

For electron microscopy, the cells were cultured on coverslips, then fixed in 0.1 M-sodium cacodylate-buffered 2% glutaraldehyde, pH 7.4, for 30 min, washed in the same buffer and post-fixed in 0.1 M-sodium cacodylate-buffered 1% osmium tetroxide, pH 7.4. After dehydration in ethanol, the specimens were embedded in Epon by inverting filled capsules over them. Ultrathin sections were stained with uranyl acetate and lead citrate, cut perpendicularly to the culture surface, examined and photographed with an Elmiskop 101 electron microscope (Siemens).

Hormone assay in incubating medium. Commercial RIA kits purchased from BioMerieux (Lyon, France) were used for determination of oestradiol and oestrone according to the manufacturer's procedures. Oestrone sulphate was measured using a specific method developed in our laboratory by Remy-Martin, Prost, Nicollier, Burnod & Adessi (1983). Briefly, free steroids were extracted from plasma with diethyl ether and steroid sulphates were isolated with use of Vlitos' reagent (methylene blue in dilute H₂SO₄/Na₂SO₄ solution). After enzymic hydrolysis, oestrone was isolated by chromatography on Celite and measured by radioimmunoassay. The intra- and inter-assay coefficients of variation were 9.7% and 10.5% respectively. No cross-reaction was found with free

oestrogens or with oestrone glucuronide. The limit of sensitivity for this technique was equal to 23.2 fmol per tube.

Oestrone and dehydroepiandrosterone sulphatase assays. After culture, the epithelial cells were removed from the flasks by scraping, centrifuged at 400 *g*, then frozen in liquid nitrogen before enzyme studies. The method for oestrone and DHEA sulphatase assays has been described previously by Prost & Adessi (1983). Briefly, the cell pellets were resuspended in 0.05 M-Tris-HCl, 0.25 M-saccharose buffer, pH 7.0, and homogenized with a Potter-Elvehjem instrument. The homogenate was centrifuged at 12 500 *g* for 20 min at 4°C. The sulphatase activities were measured in 0.05 M-Tris-HCl buffer, pH 7.6, containing [³H]oestrone sulphate (sp. act. 34 nmol/ml; 24 × 10³ d.p.m./nmol) or [³H]DHEA sulphate (sp. act. 60 nmol/ml; 15 × 10³ d.p.m./nmol) in a final volume of 0.5 ml. The reaction was started by adding the 12 500 *g* supernatant (0.10 mg protein per assay) and stopped after 30 min at 37°C by adding 1 ml 0.1 M-Na₂CO₃. [¹⁴C]Oestrone or [¹⁴C]DHEA (2000 d.p.m.) was added to correct methodological losses and unconjugated oestrone and DHEA were extracted with 5 ml diethyl ether or 4 ml petroleum benzine GR (Merck) respectively. Controls without enzyme preparation were processed simultaneously, and the minute amount of radioactivity recovered in the unconjugated fraction was subtracted from that of the experimental assay. One unit of sulphatase activity was defined as the quantity of the enzyme preparation producing one pmol of product per minute per mg DNA. The analytical criteria for oestrone and DHEA sulphatase activities in endometrium have been previously reported (Prost & Adessi, 1983). Precision values were 6.0% and 11.3% respectively. The assay blank (non-specific hydrolysis) was < 1% of the incubated radioactivity.

The DNA assay was performed according to the method of Burton (1956).

Dextran-charcoal assays of cytosolic oestradiol and progesterone receptors. The method for receptor assays has been described previously by Moutaouakkil *et al.* (1984). The epithelial cell pellets were homogenized in ice-cold buffer composed of 10 mM-Tris-HCl, 1.5 mM-EDTA, 0.5 mM-dithiothreitol, 10 mM-sodium-molybdate and 10% glycerol (v/v) (TEDM-glycerol buffer, pH 7.4). The homogenate was centrifuged at 4°C at 105 000 *g* for 60 min, using a Beckman model L8-55 ultracentrifuge equipped with a 70-1 Ti rotor. The supernatant was diluted with TEDM-glycerol to a final concentration of ~2 mg protein per ml. For oestradiol receptors, the aliquants of cytosol were incubated for 1 h at 0°C with 5 nM-[³H]oestradiol-17β, with or without a 100-fold excess of unlabelled diethylstilboestrol. Bound and free oestradiol-17β were separated by adding 200 μl of ice-cold dextran-coated charcoal suspension in TEDM-glycerol buffer, followed by an incubation of 30 min with constant stirring. After centrifugation at 4050 *g* for 10 min at 0°C, an aliquant of supernatant was used to measure the radioactivity.

A similar procedure was used for the determination of progesterone binding sites: 20 nM-[³H]R 5020 served as radioactive ligand and unspecific binding was measured by adding a 100-fold excess of unlabelled R 5020. After addition of 10 μM-cortisol and incubation for 4 h at 0°C with constant stirring, the aliquants were treated by the charcoal-dextran solution and centrifuged at 4050 *g* for 10 min at 0°C, then an aliquant of supernatant was used to measure the radioactivity. The assay of receptors was made in triplicate for each culture sample because the amount of available proteins was not sufficient to establish Scatchard plots. Negative controls with intestinal tissue demonstrated the specificity of [³H]oestradiol-17β and [³H]R 5020 binding to hormone receptors. When the values were below 10 fmol per mg protein the assay was judged as negative. The intra- and inter-assay coefficients of variation were 4.3% and 8.6% respectively (*n* = 12).

Free and sulphoconjugated steroid assays by gas chromatography-mass spectrometry. The method has been described previously by Adessi *et al.* (1982). The mass-spectrometer used was a RIBER R 10-10 B (Ribermag, Rueil, France) coupled with a gas-chromatograph GIRDEL 30 (Girdel, Puteaux, France) equipped with a SE-30 0.2 mm open tubular fused-silica capillary column.

Results

Morphological results

The appearance and growth characteristics of the two cell types in culture were studied. Phase-contrast microscopy revealed a good separation and isolation of the epithelial from the stromal cells. The epithelial component comprised glandular structures composed of several hundred cells ($10\text{ }\mu\text{m}$ in diameter) (Fig. 1a), whereas the stromal component was composed of isolated cells ($5\text{--}15\text{ }\mu\text{m}$ in diameter) which attached to the plastic surface within 30 min (Fig. 1b).

Fig. 1. Phase-contrast microphotographs of the two endometrial cell types in culture. $\times 200$. (a) Epithelial gland immediately after cell isolation; (b) stromal cells immediately after cell isolation; (c) an epithelial gland adhering to the bottom of the flask after 48 h in growth medium; (d) stromal cells at 48 h; (e) epithelial cells growing as monolayers composed of cohesive groups of polygonal cells on Day 5.

During the first 48 h after plating, the epithelial glands adhered progressively to the bottom of the flasks (Fig. 1c). The plating efficiency seemed to depend on the integrity of the glands after digestion by collagenase. The morphology of the stromal cells was mostly fibroblastic and they rapidly reached confluence in all the flasks (Fig. 1d).

After 5 days, the epithelial cells grew as monolayers composed of cohesive groups of polygonal cells (1.3×10^5 cells/cm²) on which our biochemical experiments were performed (Fig. 1e).

To confirm their structural appearance, epithelial and stromal cell cultures were examined after 5 days *in vitro* by scanning and transmission electron microscopy respectively. In the scanning electron microscope study, the epithelial cells appeared cohesive, with a circular nucleus and microvilli were scattered over their surface (Fig. 2a). The stromal cells were elongated, and large intercellular spaces were seen between the cells (Fig. 2b).

In ultrathin sections, cut perpendicularly to the monolayer surface, the shape of the epithelial cells appeared pyramidal, with a centrally located nucleus, and cytoplasmic cell processes extending peripherally (Fig. 2c). In places, the adjacent cells contacted each other by means of membrane interdigitations. Numerous microvilli were present at the upper surface of the cells; their cytoplasm contained few mitochondria, numerous polysomes and an abundant rough endoplasmic reticulum, while their nuclei appeared circular with a condensed chromatin and active nucleoli. These morphological results showed that the endometrial specific differentiation occurred in the epithelial cells cultured in the presence of oestradiol-17 β .

The cells recovered in the supernatant obtained after centrifugation of the product of endometrium digestion by collagenase displayed a typical fibroblastic morphology in culture (Fig. 2d). These cells were elongated in shape and grew as a monolayer or multilayered sheaths. Their cytoplasm showed a relative paucity of organelles, but was filled with glycogen granules, microfilaments and lipid droplets. Their nucleus was elongated with a peripheral condensed chromatin.

These results demonstrated that it is possible to culture separately epithelial and stromal cells from guinea-pig endometrium. These cells remain recognizable as uterine cells at the microscopic and ultrastructural levels by their appearance and structure. For 39 assays of epithelial cell culture, 36 yielded a monolayer cell culture after 5 days.

Metabolism of oestrogens by epithelial cells in culture

To test the effect of oestradiol-17 β on endometrial cells, it was important to obtain viable cells in an oestrogen-free culture medium.

After 5 days of culture in the presence of 10^{-9} M-oestradiol-17 β the growth medium was replaced by the harvest medium devoid of oestradiol-17 β which was changed every day for 3 days and the concentrations of oestradiol-17 β , oestrone and oestrone sulphate were measured in fresh medium and in harvest medium recovered on Days 1, 2 and 3 of the culture.

The results are reported in Table 1. The concentrations of oestrogens in the fresh harvest medium were very low and were considered as the control values. On Day 1, due to release of endogenous oestrogens from the cultured cells, the highest values were found. On Day 3, the steroid values had returned to the control levels. Identical values were obtained with an assay performed on Day 4, or when 2% Ultrosor SF was used instead of 2.5% horse serum. However, after 3

Fig. 2. Ultrastructural observations on the two types of endometrial cells after 5 days culture in growth medium. (a) Scanning electron micrograph of epithelial cells, $\times 1400$; (b) scanning electron micrograph of stromal cells, $\times 1400$; (c) transmission electron micrograph of an epithelial cell, $\times 5000$; (d) microvilli, $\times 26\,000$; (e) transmission electron micrograph of stromal cells, $\times 5000$; (f) glycogen granules and microfilaments, $\times 19\,000$.

days in the harvest medium containing 10% dextran-charcoal-pretreated FCS, oestrogen concentrations remained higher than in controls. The metabolism of oestradiol-17 β was studied in cells which had been grown for 5 days in growth medium then for 3 days in an oestrogen-free medium; these epithelial cells were then cultured for 3 more days in a fresh medium supplemented with 2.5% horse serum and 2×10^{-9} M-oestradiol-17 β . Oestrone, oestrone sulphate and oestradiol-17 β concentrations were measured in fresh harvest medium containing 2×10^{-9} M-oestradiol-17 β , and in the same medium in which the cells had lived during 3 days. As shown in Table 2, in 8 separate experiments, the oestrone concentration in the medium on Day 3 had significantly increased ($P < 0.01$) whereas the increased level of oestrone sulphate was less significant ($P = 0.05$). The release of oestrone and oestrone sulphate in the medium demonstrated that oestradiol-17 β was metabolized by epithelial cells in culture, and that 17 β -hydroxysteroid dehydrogenase and sulphotransferase activities persisted. Steroid sulphatase is the third enzyme involved in oestrogen metabolism (Adessi *et al.*, 1981). To test its activity, the oestrone and DHEA sulphatases in cultured endometrial cells were measured before and after 3 days of incubation with 2×10^{-9} M-oestradiol-17 β . Before incubation in the presence of oestradiol-17 β , the sulphatase activities (pmol product/min per mg DNA) were 211.5 (16.3–657) for oestrone sulphatase and 36.7 (5.4–69.8) for DHEA sulphatase [mean (extreme values)]. After 3 days of culture with 2×10^{-9} M-oestradiol-17 β , these values reached 177.2 (19.4–565.3) and 62.9 (7.5–204.1) respectively.

An important point was to test whether the oestrone sulphatase activity was able to hydrolyse oestrone sulphate in our cell culture conditions. For that purpose, epithelial cells were cultured in fresh medium containing 2.5% horse serum and 10^{-7} M-oestrone sulphate for 3 days, and renewed each day. On Day 3, the unconjugated and conjugated oestrogens were extracted and isolated from the culture medium, then identified by gas chromatography-mass spectrometry. Under these conditions, free oestrone, oestradiol-17 β and oestradiol sulphate were identified in the culture medium (Fig. 3).

Effects of oestradiol-17 β on cytosolic progesterone receptor amounts in epithelial cell culture

A monolayer of epithelial cells was obtained after 5 days in growth medium; this experiment

Table 1. Oestrogen concentrations in culture medium

	Oestradiol-17 β (nmol/l)	Oestrone (nmol/l)	Oestrone sulphate (nmol/l)
Fresh harvest medium	0.030 $\pm$ 0.014 (8)	0.004 $\pm$ 0.003 (8)	0.090 $\pm$ 0.014 (8)
On Day 1	0.100 $\pm$ 0.020 (8)	0.030 $\pm$ 0.010 (8)	0.160 $\pm$ 0.007 (8)
On Day 2	0.050 $\pm$ 0.010 (8)	0.010 $\pm$ 0.007 (8)	0.120 $\pm$ 0.007 (8)
On Day 3	0.030 $\pm$ 0.007 (8)	0.004 $\pm$ 0.003 (7)	0.070 $\pm$ 0.014 (8)

Values are mean $\pm$ s.d. for the no. of observations in parentheses.

Table 2. Oestrogen metabolism in epithelial cell culture

	Oestradiol-17 β (nmol/l)	Oestrone (nmol/l)	Oestrone sulphate (nmol/l)
Fresh harvest medium with 2×10^{-9} M- oestradiol-17 β	2.030 $\pm$ 0.014 (8)	0.004 $\pm$ 0.003 (8)	0.090 $\pm$ 0.014 (8)
On Day 3	1.420 $\pm$ 0.113 (8)	0.100 $\pm$ 0.010 (8)	0.190 $\pm$ 0.040 (8)

Values are mean $\pm$ s.d. for the no. of observations in parentheses.

Fig. 3. Identification after 3 days by mass spectrometry of unconjugated and conjugated oestrogens (a, oestrone; b, oestradiol-17 β) present in the culture medium, renewed each day, and containing 10^{-7} M-oestrone sulphate. The identification of oestradiol-17 β in the sulphate fraction reveals the presence of oestradiol sulphate.

required $2.3 \pm 0.1 \times 10^7$ cells per assay. The oestradiol induction was studied in the epithelial cells obtained after 3 days in oestrogen-free medium and the fluid was changed on each of the 3 days. These cells were then incubated in fresh medium containing 2.5% horse serum and 2×10^{-9} M-oestradiol-17 β for 3 days. The cells were harvested and homogenized and the amounts of cytoplasmic oestradiol and progesterone receptor were determined by dextran-charcoal assays. Each value represents the average of 6 experiments. Before oestradiol-17 β induction, the cytosolic receptor values were 3.58 ± 1.34 and 3.60 ± 1.00 fmol/ μ g DNA for progesterone and oestradiol respectively. The addition of oestradiol-17 β produced an increase in progesterone receptor values to 7.63 ± 1.10 fmol/ μ g DNA ($P < 0.05$). The oestrogenic stimulation of the cytosolic progesterone receptors corresponded with a decrease in the cytosolic oestradiol receptor values (to 2.37 ± 0.57 fmol/ μ g DNA).

Discussion

The present study describes a simple and original method for culturing guinea-pig endometrium as separate epithelial and stromal components. The primary epithelial cell cultures described above provide a suitable system for the study of oestrogen metabolism and oestrogen-induced responses.

We have tested two different methods for endometrial cell component isolation. The observations by phase-contrast microscopy, during cell dissociation, show that the method of successive centrifugation described by Varma *et al.* (1982) is more efficient than that of filtration across a 38 µm stainless-steel grid used by Fleming & Gurpide (1981). The integrity of the glands after digestion by collagenase is better preserved in the first method and the plating efficiency seems to depend on this integrity. If necessary, after separation the pooled gland fraction may be purified from contaminating stromal cells as follows: the epithelial clumps are placed in flasks in culture medium and then incubated at 37°C in a 95% air–5% CO₂ atmosphere; the residual stromal cells attach selectively to the plastic within 30–45 min, leaving the epithelial glands floating in the medium. The purified epithelial glands are then collected and plated, as described by Kirk & Irwin (1980).

On Day 5 of the culture, phase-contrast microscopy was used to distinguish the cell types. The flasks containing each type of cells were examined daily. The efficiency of epithelial cell growth in culture seemed to be improved by simple preincubation of the flasks with growth medium which allows the epithelial glands to adhere more quickly. However, the stromal cells attach to the plastic surface more readily and have a fibroblastic appearance. Comparable results have been reported for the rat by Echeverria *et al.* (1980) and McCormack & Glasser (1980), for the rabbit by Ricketts *et al.* (1983) and for the human by Varma *et al.* (1982). Our preliminary assessment of the two different endometrial components on Day 5 was completed by their characterization with scanning electron microscopy. The features defining the two endometrial cell types are as follows: in epithelial cell culture, the monolayer is composed of polygonal cells with numerous microvilli and a round nucleus, whereas the stromal cells display a typical fibroblastic appearance. Such cultures as we obtained can be clearly categorized as of epithelial or mesenchymal origin. Even at the ultra-structural level, the epithelial cells *in vitro* display a plasma membrane with microvilli, cytoplasm with electron-dense granules, numerous microtubes and polysomes, abundant rough endoplasmic reticulum and an active nucleus in which the chromatin was mostly extended. In culture, these cells show structural features remarkably similar to those described for endometrial epithelial cells *in situ*, whereas the stromal cells appear as typical fibroblasts. The third cell type described by Varma *et al.* (1982) for human endometrium (displaying epithelial cell characteristics by phase-contrast microscopy, but mesenchymal characteristics of stromal cells by transmission electron microscopy) has not been described for the rat (Echeverria *et al.*, 1980), the rabbit (Ricketts *et al.*, 1983) or guinea-pig (present study).

In our study, an important condition was to ensure the complete removal of endogenous steroids before oestrogen stimulation. This is why fresh medium containing 2.5% horse serum was added on 3 consecutive days; the oestrogen content of horse serum is below the limit which elicits an epithelial cell response (Lee, Davies, Soto & Sonnenschein, 1981). FCS pretreated by dextran-charcoal has been used for preliminary experiments, but the residual oestrogen level was higher than the threshold of response of the epithelial cells. We have used the steroid-free serum substitute Ultrosor SF which is as efficient as horse serum in maintaining healthy cells. After complete removal of endogenous steroids, the incubation with 2×10^{-9} M-oestradiol-17β for 3 days shows the persistence of the normal oestrogen metabolism in guinea-pig epithelial cell culture. Indeed, the oestrogen assays after incubation show a conversion of oestradiol-17β to oestrone due to the presence of the 17β-hydroxysteroid dehydrogenase still active in cells. Moreover, the oestrone sulphate assay demonstrates that a sulphotransferase activity is also present in cell culture, which converts oestrone into a sulphoconjugated derivative. The oestrone and DHEA sulphatase activities previously characterized by Moutaouakkil *et al.* (1984) in the uterus and liver of female guinea-pigs have been demonstrated and measured in the epithelial cell culture. The three enzymes

interfering with oestrogen metabolism are present in our epithelial cell culture and their patterns in epithelial cells cultured *in vitro* resembled that of fresh endometrium.

The induction of cytosolic progesterone receptors by oestrogen has been studied in rat uterus (Kassis, Sakai, Walent & Gorski, 1984), in guinea-pig fetal uterus in organ culture (Sumida *et al.*, 1983) and in human endometrial cell culture (Eckert & Katzenellenbogen, 1981). Our studies indicate that the stimulation of cytosolic progesterone receptor content in primary cultures of guinea-pig endometrium epithelial cells is a sensitive indicator of its responsiveness to oestradiol-17 β . In our experiments, the magnitude of the response varied from 1.3 to 10.8 times and oestradiol-17 β promoted an apparent dose-dependent depletion of cytosolic oestrogen receptors which may correspond to the accumulation of nuclear oestrogen receptors (Eckert & Katzenellenbogen, 1981) or to a greater retention of the steroid-receptor complex in the nucleus (Gorski, Welshons & Sakai, 1984; Stack & Gorski, 1985). The mass spectrometry study carried out on the culture medium after incubation with oestrone sulphate showed the presence of oestrone, oestradiol-17 β and oestradiol sulphate, confirming previous studies. Briefly, extracellular oestrone sulphate is hydrolysed by plasma membrane-bound sulphatase and enters into epithelial cells as unconjugated oestrone. In the endometrial cell, the action of 17 β -hydroxysteroid dehydrogenase leads to the synthesis of oestradiol-17 β (Adessi *et al.*, 1982). Conversely, a soluble sulphotransferase converts intracellular free oestrogens into their conjugated derivatives which are inactive metabolites (Adessi *et al.*, 1982). Moreover, the conversion of oestrone sulphate to oestradiol sulphate must arise without prior removal of the sulphate moiety and not by hydrolysis of the oestrogen sulphates followed by resulphurylation (Adessi *et al.*, 1981).

In conclusion, the procedure and results described here for the preparation of primary cultures of the epithelial cells of guinea-pig endometrium provide a system which can be used to study hormone effects on protein synthesis in hormone-responsive endometrial cells in culture.

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