Human Endometrial Cancer Cell Cultures for Hormonal Studies

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Abstract

A cell line of human endometrial adenocarcinoma (HEC-1), established by Kuramoto et al. in 1968, was studied. The presence of estradiol receptors in these cells was documented. The receptors were found to reside mainly in the nucleus, even when cells were grown in estrogen-free medium. Isolated nuclei from HEC cells could be labeled during incubations with [3H]estradiol at 0-4°C. The induction of estradiol 17β-dehydrogenase by progestins, which has been demonstrated to occur in vivo and in vitro in normal proliferative endometrium, was attempted with these cells. The attempts were unsuccessful both when estradiol and medroxyprogesterone acetate were added to the culture medium and when they were injected into hamsters bearing adenomatous tumors resulting from the transplantation of HEC cells into the cheek pouch.

Intact glands, free of stromal components, were obtained by collagenase treatment of specimens of well-differentiated endometrial carcinoma, followed by filtration through stainless steel sieves. An abundance of branched glands was evident by light microscopy. Epithelial cell monolayers were derived from these glands and examined by light and electron microscopy.

The preparation of glands and epithelial cell monolayers from specimens of endometrial carcinoma may provide opportunities to evaluate the responsiveness of the neoplasm to progestins, a test of potential value for the prediction of the efficacy of progestin treatment in individual patients. This approach may obviate the problems of viability encountered during the maintenance of fragments of carcinomatous tissue in organ culture.

Introduction

Progestins administered in large doses produce remissions of metastatic endometrial cancer in about one-third of the treated patients (2, 10, 17). The effectiveness of this form of therapy may be related to the antiestrogenic actions of progestins in the endometrium. The mechanism by which the actions of estradiol are inhibited in this tissue involves at least 2 effects of the progestins: reduction of estrogen receptor levels (23) and induction of estradiol 17β-dehydrogenase (22). Dependence of hormonal action on receptor levels is a well-accepted concept. An elevation of the enzyme involved in the conversion of estradiol to estrone results in a lowering of the intracellular level of estradiol and, consequently, in a depression of the action of the hormone on the endometrium (21).

On the basis of these observations, in vivo tests for the prediction of responsiveness of individual patients to progestin therapy have been devised. A decline in the estradiol receptor levels and an increase in estradiol 17β-dehydrogenase activity in biopsies of endometrial carcinoma was noted to occur in some patients after the administration of progestins for a few days (8, 16, 24). These results, however, have not been correlated with clinical responses to long-term hormonal treatment.

The development of an in vitro predictive test based on the induction of estradiol 17β-dehydrogenase by progestins added to the medium in which biopsy fragments are maintained in organ culture for 1 to 2 days would be desirable. The feasibility of this approach was suggested by the observation that severalfold increases in the enzymatic activity can be consistently obtained with normal proliferative endometrium (22). However, carcinomatous tissue appears to be less viable under culture conditions, and a decline in activity in the control dishes is regularly observed. Attempts are currently being made to modify the incubation medium to provide factors that may be needed by the neoplastic tissue to preserve its viability and capacity to respond to inducers in vitro.

It could be expected that studies with endometrial cancer cells grown and maintained in culture for prolonged periods would eliminate problems of viability and serve to identify the requirements for hormonal responses. Accordingly, the available HEC-1 (human endometrial cancer) cell lines were examined to determine whether they contained estrogen and progesterone receptors and whether they were responsive to hormonal influences.

Since studies with any particular endometrial cancer cell line are necessarily biased by the characteristics of the original source, a procedure for the separation and growth of glandular and stromal elements of specimens of endometrial cancer, taken from patients treated at our institution, was developed. The originality of the procedure resided in the isolation of glands, free from stromal elements, by filtration through sieves after mild digestion with collagenase. These preparations may allow comparisons of the biochemical characteristics of epithelial cells from individual patients with cancer of the endometrium and from normal subjects under similar experimental conditions.

Materials and Methods

HEC Cells in Culture

HEC-1A and HEC-1B cells for starting cultures were kindly provided by Dr. J. Fogh, Sloan-Kettering Institute for Cancer Research. The original cell line (HEC-1A) was established in 1968 by Kuramoto et al. (12) from explants of moderately differentiated papillary adenocarcinoma ob-
tained from a 71-year-old woman. A subline (HEC-1B) was obtained by Kuramoto (11) from a group of cells that sustained a stationary period of 135 to 190 culture days. The HEC-1A cells have been reported to be predominantly diploid, and the HEC-1B subline is predominantly tetraploid.

These 2 cell lines have been kept in continuous culture in our laboratory since 1976 in T75 flasks (Corning Glass Works, Corning, N. Y.), as well as in Roller bottles (New Brunswick Scientific Co., New Brunswick, N. J.), in McCoy's 5a medium (Grand Island Biological Co., Grand Island, N. Y.) containing 10% calf serum or fetal calf serum (Flow Laboratories, Rockville, Md.), 10 μg insulin (Eli Lilly and Co., Indianapolis, Ind.) per ml, and 1% antibiotic-antimycotic mixture (Grand Island Biological Co.). Three days prior to using the cells for estrogen receptor studies, we replaced the culture medium with a medium of the same composition but containing calf serum pretreated with dextran-coated charcoal (18 mg/ml serum) for 30 min at 37°C. We replaced the culture medium with a medium of the same composition but containing calf serum pretreated with dextran-coated charcoal (18 mg/ml serum) for 30 min at 37°C to remove endogenous steroids.

Cultures in dishes or flasks were carried out in a humidified atmosphere of 5% CO2 and 95% air in a National CO2 incubator at 37°C. Roller glass bottles were tightly closed after equilibration with CO2:O2 (5:95) mixture and kept rotating on a Rollacell apparatus (New Brunswick Scientific Co.) placed in an environmental room at 37°C.

Cells were removed from culture flasks by treatment with 0.05% trypsin:0.02% EDTA in Ca2+- and Mg2+-free Hank's balanced salt solution (Grand Island Biological Co.) and from Roller bottles by treatment for 10 min at 37°C with 1 mM EDTA in Dulbecco's phosphate-buffered saline, Ca2+ and Mg2+ free (Grand Island Biological Co.).

**Specific Estradiol Binding**

The presence of estrogen receptors in HEC cells was investigated by the incubation of [6,7-3H]estradiol (48 Ci/mmol) (New England Nuclear, Boston, Mass.) with either whole cells, cytosol, or isolated nuclei. Approximately 2 x 107 cells, cultured for 3 days in medium prepared with charcoal-treated serum, were used for each experiment.

**Whole-Cell Labeling**. HEC-1A and HEC-1B cells were washed with Dulbecco's phosphate-buffered saline, Ca2+ and Mg2+ free, resuspended in Earl's balanced salt solution (Grand Island Biological Co.) and incubated with 20 nM [3H]estradiol (approximately 1.5 x 106 cpm/ml) for 30 min at 37°C. Parallel incubations were conducted in the presence of 2 μM DES. Labeled cells were washed with Earl's balanced salt solution, resuspended in TED buffer, and homogenized over ice in a 2-ml glass homogenizer (Ace Glass Co., Vineland, N. J.).

Nuclei were separated by centrifugation at 800 x g for 10 min, washed with TED buffer, resuspended in 0.5 ml TED buffer:0.5 mM KCl, and kept in this buffer for 1 hr at 0-4°C, with occasional stirring. After centrifugation at 2500 x g for 15 min, the supernatant ('nuclear extract') was subjected to ultracentrifugation at 166,000 x g for 16 hr in a 10 to 30% discontinuous sucrose density gradient prepared in 10 mM Tris, pH 7.8; 1 mM EDTA; and 1 mM KCl. Fractions (0.2 ml) were collected, mixed with Scintiverse (Fisher Scientific, Westford, Mass.), and counted in a liquid scintillation spectrometer (Liscop; Nuclear-Chicago Corp., Des Plaines, Ill.) to determine the distribution of radioactivity.

The supernatant of the nuclear pellet obtained by centrifugation of the cell homogenate was centrifuged at 105,000 x g for 1 hr to obtain about 1 ml cytosol containing approximately 5 mg protein per ml. Samples of cytosol were examined by ultracentrifugation following the procedure described above but with a 5 to 20% sucrose gradient in 10 mM Tris, pH 7.8:1 mM EDTA:0.5 mM KCl buffer.

In some tubes a solution of bovine serum albumin labeled by acetylation with [14C]acetic anhydride was used instead of the sample and served as reference for the estimation of sedimentation constants.

**Cytosol Labeling**. Cytosol of unlabeled HEC-1A or HEC-1B cells was obtained as described above. A 20 mM solution of [3H]estradiol in 1 ml cytosol (about 1.5 x 106 cpm/ml) was prepared and kept at 0-4°C for 2 hr. The solution was then treated with dextran-coated charcoal for 15 min at 0-4°C to remove unbound [3H]estradiol. Aliquots of labeled cytosol were then subjected to sucrose gradient ultracentrifugation under either "high-salt" or "low-salt" conditions.

The high-salt gradient was 5 to 20% sucrose in 10 mM Tris:1 mM EDTA:0.5 mM KCl, and the low-salt gradient was 10 to 30% sucrose in 10 mM Tris:1 mM EDTA:1 mM KCl. Parallel analyses were performed in the presence or absence of DES. In these experiments bound and unbound radioactivity was separated by adsorption on dextran-coated charcoal at 0-4°C for 15 min. The data were analyzed according to Scatchard (5).

**Isolated Nuclei Labeling**. Nuclei from unlabeled HEC-1B cells were obtained as described above, either with the use of TED buffer or a 0.32 mM sucrose:10 mM Tris:1 mM MgCl2:1 mM dithiothreitol buffer, which yielded morphologically less altered nuclear preparations. Nuclei were resuspended in the corresponding buffer solution made 2 to 25 nM in [3H]estradiol, and the suspension was kept overnight at 4°C. Labeled nuclei were then washed once with 0.1% Triton X-100 in 0.1 mM Tris buffer and twice with 0.1 mM Tris buffer. The washed pellet was mixed with ethanol; the ethanol solution was used for measurement of radioactivity, and the precipitate was used for measurement of DNA by the method of Burton (4).

**Stability of Progesterone and MPA in HEC Cell Cultures**

For the determination of the stability of progesterone and MPA (The Upjohn Co., Kalamazoo, Mich.) in HEC cell cultures, isotopically labeled compounds were added to HEC-1B cultures growing in T-75 flasks, and samples of medium were taken at 0, 1, 2, 4, 6, and 24 hr after the addition. As control for losses of labeled compounds by adsorption to the container, T-75 flasks containing medium but no cells were used. [4,14C]Progesterone (55.7 mCi/mmol) and [1,2-3H]MPA (50 Ci/mmol), both supplied by New England Nuclear, were used for these tests after verification of radiochemical homogeneity. Initial concentrations of the labeled compounds in the medium were
about 20,000 cpm of [14C]progesterone per ml (approximately 100 ng/ml) and 200,000 cpm of [3H]MPA per ml (approximately 1 ng/ml). Samples of medium (0.5 ml) were extracted with 3 ml of ethyl acetate containing 100 µg of carriers (MPA or progesterone plus 20α dihydroprogesterone), and the extract was subjected to thin-layer chromatography on silica gel thin-layer plates (GF; Analtech, Inc., Newark, Del.) with the solvent system chloroform:acetone:hexane, 4:1:3. After localization of the carriers by UV absorption, the bands corresponding to progesterone and MPA were eluted with ethyl acetate and counted in a liquid scintillation spectrometer. Recoveries of the added carriers were determined by measuring absorbance at 240 nm in aliquots of the eluted material.

Transplantation of HEC Cells into the Cheek Pouch of Hamsters

About 10^6 to 10^7 cells were transplanted into the cheek pouch of female hamsters weighing approximately 50 g under Nembutal anesthesia. The animals received cortisone acetate i.m. (2.5 mg/50 g body weight) at the time of transplantation and every fourth day thereafter. When tumors became palpable (between Days 16 and 20), some of the hamsters were treated with estradiol and MPA for study of the hormonal effects on the activity of estradiol 17β-dehydrogenase in the transplanted cells, as described below.

Exposure of Cells in Culture or Tumors in Vivo to Estradiol and MPA

To study the effects of MPA on the levels of estradiol 17β-dehydrogenase, we grew HEC cells in medium containing MPA (500 ng/ml; 1.3 x 10^-6 M), either alone or in combination with estradiol (5 ng/ml; 1.8 x 10^-6 M). Other cultures were exposed to only estradiol or to estradiol for 1 day and a mixture of estradiol and MPA, at the concentrations indicated above, for 1 to 3 days.

Hamsters with HEC tumors were treated with 1 µg estradiol s.c. daily for 2 days and then with MPA (Depo Provera; The Upjohn Co.) in 0.9% NaCl solution, 1 mg s.c. daily for 2 days. Controls received only estradiol.

Estradiol 17β-Dehydrogenase Assay

For determination of estradiol 17β-dehydrogenase activities, cultured cells, centrifuged at low speed and washed twice with 5 ml Hanks' balanced salt solution, or tumors excised from the hamster cheek pouch were homogenized at 4° in a glass homogenizer with 50 mM Tris, pH 8.0, containing 1.4 µM NAD^+ to obtain a protein concentration of about 1 mg/ml. The homogenates were centrifuged at 800 x g for 10 min, the supernatants were transferred to 12- x 75-mm assay tubes, and the tubes were immersed in a shaker water bath at 37°. The reaction was initiated by the addition of a solution of [3H]estradiol (specific activity, 300 cpm/pmol) in ethanol to obtain a 20 µM final concentration (6 x 10^6 cpm/ml). The ethanol concentration in the assay mixture was kept below 1%. Aliquots (0.1 ml) were taken at 1, 3, 6, and 9 min. Each aliquot was immediately mixed with 2 ml of methanol containing 1000 cpm of [14C]estrone, 500 µg of unlabeled estrone, and 500 µg of unlabeled estradiol. The methanolic solutions were evaporated to dryness, and the residues were chromatographed on silica gel thin-layer plates (GF; Analtech, Inc.) with the system chloroform-ethyl acetate, 4:1. In each chromatogram the UV-absorbing band corresponding to estrone was eluted with ethyl acetate, and the H2:14C ratio in the eluate was determined in a liquid scintillation spectrometer. Aliquots (0.1 ml) of the supernatant used for the assay were taken for protein determination by the method of Lowry et al. (13) with bovine serum albumin as standard.

The concentration of [3H]estrone in the aliquot taken from the reaction mixture was calculated as follows:

\[ \frac{[3H]estrone (nmol/mg protein)}{[3H]:14C in estrone (cpm/cpm)} \times \frac{specific activity of [3H]estradiol (cpm/pmol)}{volume of sample (ml) \times concentration of protein (mg/ml)} \]

Rates were estimated by plotting these values versus time of sampling and determining the line best fitting these points by least squares. Estradiol dehydrogenase activities were expressed as nmol of estrone formed from estradiol per mg of protein per hr.

Isolation of Glands from Specimens of Endometrial Cancer

Curettages of well-differentiated adenocarcinoma were taken from uteri of postmenopausal women undergoing hysterectomy. The tissue was transported to the laboratory in sterile vials with growth medium (McCoy's 5a medium containing 10% fetal calf serum; insulin, 10 µg/ml; penicillin, 100 units/ml; streptomycin, 100 µg/ml; and Fungizone, 2 µg/ml). After removal of blood clots, the tissue was cut into approximately 1-cm pieces and placed in sterile vials containing 0.25% collagenase (Type I; Worthington Biochemical Corp., Freehold, N. J.) in the same medium. The capped vials were kept in a water bath at 37°, with shaking, for 2 hr. The contents of the vials were strained through a 250-µm stainless steel sieve (Millipore Corp., Bedford, Mass.), and the filtrate was then passed through a 105-µm stainless steel grid (Cistron Corp., Lebanon, Pa.). The glands retained after the second filtration were washed with about 50 ml of Ca^2+- and Mg^2+-free Hanks' balanced salt solution containing 2% of the antibiotic-antimycotic mixture (Grand Island Biological Co.). The washed glands were transferred to a culture dish by backwashing with 20 to 40 ml of the growth medium, distributed into several culture dishes (10 cm in diameter; Falcon Plastics Co., Oxnard, Calif.), and examined with an inverted microscope.

Epithelial Cell Cultures from Endometrial Glands

The dishes with the glands were placed in the incubator at 37° in a humidified 5% CO2:95% air atmosphere, and within 1 day epithelial cells were seen spreading from the glands and growing in a monolayer. For up to 2 weeks, a period during which the cells were actively proliferating, growth medium was added to the dishes every 3 days without removing the old medium. After this period the
medium was renewed twice a week. When the cells reached confluence, they were transferred to T-25 flasks. Cultures have been extended to 60 days.

Morphological Studies

Samples of isolated glands were fixed for 5 hr in 0.1 M phosphate-buffered 4% glutaraldehyde (pH 7.4) at 4°. The tissues were washed and left in buffer at 4° overnight. The following morning they were further fixed in buffered 1% osmium tetroxide. The tissues were dehydrated through a graded series of ethanol and propylene oxide and embedded in Epon.

Monolayers growing in culture were examined daily by phase-contrast microscopy. Upon termination of the culture, the medium was decanted, and the monolayers were washed with buffer, processed directly in the culture dishes as with the isolated glands, and embedded in Epon by inverting filled BEEM capsules over them. One-μm sections stained with toluidine blue were examined by light microscopy. Gray to silver thin sections of the monolayers were stained with uranyl acetate and lead citrate, examined, and photographed in a JOEL 100B electron microscope at 60 kV.

Results

Estradiol Receptors in HEC Cells. Charts 1 and 2 show sedimentation patterns in sucrose gradients obtained with a nuclear extract and a cytosol preparation of HEC-1A cells prelabeled with [3H]estradiol. A single peak of radioactivity located in the 3S to 4S region was noted in both the nuclear and cytosol samples. Binding of [3H]estradiol to the macromolecules sedimenting in this region was practically eliminated by DES, as is characteristic of tissue estrogen receptors. The amounts of receptor-bound radioactivity in nuclear preparations were greater than those found in cytosol, as would be expected, since most of the cytoplasmic receptor should have translocated to the nucleus under the labeling conditions used.

Scatchard analysis of the interaction of [3H]estradiol with cytosol binders in HEC-1B cells showed a concentration of binding sites of about 50 fmol/mg protein and a dissociation constant of about 5 nM. Lower receptor levels (about 10 fmol/mg protein) but similar dissociation constants were found when cytosol from HEC-1A cells was used.

The amounts of estradiol receptor found in nuclear extracts after incubations of whole cells with [3H]estradiol appeared to be much greater than expected from the receptor levels measured in cytosol preparations from cells cultured for several days in the absence of estradiol. It was therefore suspected that, even under these culture conditions, estrogen receptors may remain in the nucleus. Chart 3 shows the results from an experiment in which nuclei isolated from HEC-1B cells grown in estrogen-free medium...
for 3 days were incubated with [³H]estradiol at 4°C overnight. These data indicate that about 1 pmol of estrogen per mg of DNA can be specifically bound to receptors in the nucleus, even at low temperatures. Similar results were obtained when nuclei were prepared and labeled with either TED buffer or the Tris:sucrose:Mg⁺²:dithiothreitol buffer.

**Metabolism of Progesterone and MPA by HEC Cells in Culture.** As shown in Table 1, progesterone added to the medium of HEC-1B cultures was rapidly metabolized (t₁/₂ ∼2 hr), whereas about 60% of the MPA remained unchanged after 24 hr of incubation.

The pattern of metabolism of progesterone during incubations with HEC-1B cells was very similar to the pattern of metabolism of the hormone incubated with fragments of normal proliferative or secretory endometrium (P. G. Satyaswaroop and E. Gurpide, unpublished results).

**Attempts to Induce Estradiol 17β-Dehydrogenase in HEC Cell Cultures and Tumors in Hamster Cheek Pouch.** The activity of estradiol 17β-dehydrogenase in HEC-1A and HEC-1B cells in culture was found to be low (about 0.3 nmol estrone per mg protein per hr). Culturing the cells for 3 to 4 days in the presence of MPA (about 10⁻⁸ M) did not change the level of enzymatic activity. No changes were observed either when mixtures of MPA and estradiol (approximately 10⁻⁸ and 10⁻⁶ M, respectively) were present in the culture medium or when the cells were exposed to estradiol for 1 day and then to the MPA:estradiol mixture for 3 days.

Tumors derived from HEC-1A and HEC-1B cells transplanted into the hamster cheek pouch also showed low estradiol 17β-dehydrogenase activity (0.4 nmol estrone per mg protein per hr), apparently not significantly different from the enzymatic activity in the transplanted cells. This level of activity was not changed by 2 daily injections of estradiol (1 μg) and MPA (1 mg).

**Morphology of HEC Tumors Developed in the Hamster Cheek Pouch.** Examination of hematoxylin and eosin-stained sections of tumors recovered from hamster cheek pouches revealed circular clusters of epithelial cells with intervening connective tissue septa. Some of the clusters contained central lumen-like spaces. In those instances the epithelium exhibited a stratified or pseudostratified arrangement.

**Morphology of Glands Isolated from Endometrial Adenocarcinoma and Monolayers Derived from Them.** Phase-contrast or light microscopy of isolated gland preparations and sections of embedded samples revealed that they consisted of tubular structures of varied lengths, composed of a simple low columnar epithelium. The isolated glandular units were devoid of extracellular components and exhibited a complex branching pattern (Fig. 1). Branched glands were also found in preparations from hyperplastic endometrium but were not as ramified (Fig. 2).

Light microscopic examination of the monolayers during the first week of culture showed that they were composed of polyhedral cells, often in close contact with each other. Arrays of linear structures were located in the attenuated peripheral cytoplasm. Granular organelles resembling lysosomes were abundant. Nuclei contained deeply stained pleomorphic nucleoli (Figs. 3 and 4).

Examination by electron microscopy revealed an elaborate meshwork of microfilaments in the ectoplasmic region of the attachment surface of the cells (Fig. 5). Microfilaments were markedly reduced in number in deeper sections of the cells. Instead, the cytoplasm contained an abundance of free ribosomes and autophagic vacuoles (lysosomes). Microvilli were present on all free surfaces of the plasma membrane. Although they varied in length from cell to cell, they were uniform in diameter. Membrane specializations were observed at points where adjacent cells came into contact. Other organelles (Golgi apparatus, rough endoplasmic reticulum, mitochondria, etc.) were present and showed no unusual features (Figs. 6 and 7).

**Discussion**

Cultures of the HEC cell line showed no responsiveness to progestins, at least in regard to the inducibility of estradiol 17β-dehydrogenase by MPA. Shapiro et al. (18) have also reported failures to affect protein synthesis in HEC cells with estrogens and progesterone.

Several reasons may be proposed for these negative results. The endometrial adenocarcinoma from which these cells originate might have been of the type unresponsive to progestins, since about two-thirds of the patients with endometrial cancer do not respond to progestin treatment. It is also possible that transformed cells lack some factors, present in normal endometrium, which are required for responsiveness and might be supplied in vivo by the circulation. The experiments with cells transplanted into the cheek pouch of the hamster, planned as one test of such a possibility, yielded clearly negative results. The adenomaous appearance of the tumors developed in the hamsters served to verify the identity of the cell line kept in culture.

The presence of progesterone receptors is probably required for hormonal activity. Unfortunately, in contrast with the clear evidence for the existence of estrogen receptors, the existence of receptors for progestins in HEC cells has not yet been adequately demonstrated. Sucrose gradient ultracentrifugation and dextran-coated charcoal adsorption methods to detect receptor-bound [³H]progesterone or [³H]R5020 (kindly provided by Dr. J. P. Raynaud, Roussel-Uclaf, Paris, France) revealed very low levels of cytoplasmic-specific binders.

The isolation of glands from specimens of endometrial cancer and the preparation of epithelial cell cultures from

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**Table 1**

**Stability of progesterone and MPA in HEC-1B cell cultures**

Labeled progesterone or MPA was added to cultures of HEC-1B cells. Samples of the medium were taken at various intervals, and concentrations of the labeled compounds were determined by procedures involving thin-layer chromatography and correction for losses incurred during isolation. The results (average of 2 experiments) are expressed in terms of percentage of the initial concentration remaining in the medium at various periods of incubation.

<table>
<thead>
<tr>
<th>Substrate</th>
<th>Initial concentration (ng/ml)</th>
<th>Amount remaining in medium after various periods of incubation (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>[¹⁴C]Progesterone</td>
<td>100</td>
<td>100, 60, 24, 10, 0</td>
</tr>
<tr>
<td>[³H]MPA</td>
<td>1</td>
<td>95, 95, 93, 93, 57</td>
</tr>
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these glands offer the opportunity for testing in vitro responses in a large number of endometrial cancers. Since similar preparations can be obtained from normal proliferative endometrium (P. G. Satyaswaroop, R. S. Bressler, and E. Gurpide, unpublished data), it will be possible to verify whether isolated glands and epithelial cell cultures retain the characteristics of the original tissue, which has consistently responded to MPA in vitro with a severalfold increase in estradiol 17β-dehydrogenase activity.

The need for measuring the extent to which the hormone under study is metabolized by the cultures is made apparent by comparison of the half-lives of the disappearance of progesterone (~2 hr) and MPA (~24 hr) from the medium in which HEC cells are grown. Degradation of insulin, reported by Osborne et al. (15) to occur in cultures of human breast cancer cells, has also been observed with the HEC cells.

Although Shapiro et al. (18) reported that HEC cells lack estradiol receptors, the results of the present study indicate that estradiol receptors are present in these cells. Probably, the method used by Shapiro et al. was less sensitive than were the methods used here.

It was of interest to find that most of the estrogen receptors in HEC cells were in the nuclear fraction rather than in cytosol, in spite of the fact that estrogens were eliminated from the culture medium 2 to 3 days before the cells were used for receptor studies. Furthermore, isolated nuclei could be labeled with [3H]estradiol at 4°, a temperature at which exchange with hypothetical receptor-bound estrogen was considered to be unlikely. These observations suggest the possibility that "unoccupied" estrogen receptor may be localized in the nucleus of HEC cells. Brooks et al. (3) and Zava and McGuire (26) have reported nuclear labeling with [3H]estradiol at 0° in the human breast tumor cell line MCF-7, and Soto et al. (20) described binding of [3H]estradiol at 4° to nuclear extracts of rat endometrial and pituitary cell lines.

Additional relevance to these findings is provided by the observation that nuclei isolated from normal human endometrium can also be specifically labeled with [3H]estradiol during incubations at 0-4° (H. Fleming and E. Gurpide, unpublished results). It was somewhat surprising not to detect a second peak of radioactivity in the 8S region during low salt sucrose gradient ultracentrifugation of HEC cytosol incubated with [3H]estradiol. Planned estrogen receptor studies will attempt the inhibition of proteolytic enzymes, which might be responsible for the formation of the 3S to 4S species at the expense of other forms of the receptor (14) and even for the presence of unoccupied receptors in the nucleus.

The techniques of isolation of glands and "whole-mount" examination utilized in this investigation provide a more suitable means of observing the 3-dimensional architecture of uterine glands than do the more conventional histological methods. Branching and budding of glands have been reported in microscopic sections of endometrium obtained from cases of adenocarcinoma and hyperplasia (7, 9, 19). It has been suggested that these features are of diagnostic value. Conventional microscopy would require reconstruction of serial sections for determination of the complexity of branching in any particular specimen. In contrast, the present method affords a quick and convenient overview of the glandular units. Indeed, differences in complexity of branching of glands obtained from hyperplastic or adenocarcinomatous endometrium are readily discerned in the present study.

Furthermore, isolation of glands provides a pure source of endometrial gland cells for the establishment of uterine cell lines. The examination of isolated glands and sections of them by light microscopy confirms the absence of stromal cell contamination.

All cells of the monolayer exhibited microvilli, a feature that in the uterus is limited to epithelial cells (25). Migrating fibroblasts in culture may show projections resembling microvilli that are associated with the ruffled border (1), but in those cases they are located only at the leading lamella of the cell and vary in appearance. In contrast, the microvilli of the cells in this study were located on all free surfaces and were of uniform diameter. The occasional spherical cells seen in culture were not well attached to the culture dish and exhibited signs of degeneration. They were interpreted to be dying cells that were rounded up and detaching from the monolayer.

The cells share a number of fine structural features with neoplastic cells in vivo [reviewed by Ferenczy (6)]. Most notable of these are an abundance of microfilaments, lysosomes, and free cytoplasmic ribosomes. However, it is not unusual to find these features in cells growing in monolayers. Therefore it is not appropriate to draw conclusions concerning their significance until similar in vitro studies of normal endometrial glands are available for comparison.

Acknowledgments

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Fig. 1. Whole-mount preparations of human endometrial glands isolated from a well-differentiated adenocarcinoma after collagenase digestion and prior to filtration. Two points of branching can be seen (arrowheads). × 300.

Fig. 2. Whole-mount preparation of human endometrial glands isolated from hyperplastic endometrium. × 300.

Fig. 3. Phase-contrast micrograph of a 2-day live culture derived from endometrial glands isolated from a well-differentiated adenocarcinoma. The monolayer consists of polyhedral cells of varying degrees of attenuation and some poorly attached spherical cells. Granular inclusions are present in the cytoplasm of some cells (arrowhead). × 1,200.

Fig. 4. Photomicrographs of 1-μm plastic sections from a 7-day culture of isolated glands derived from a well-differentiated adenocarcinoma, cut parallel to the attachment surface of the cells. The cells contain dense, stained pleomorphic nuclei and an abundance of clear vacuolar spaces in the cytoplasm. Toluidine blue, × 1,200.

Fig. 5. Electron micrograph of a section of a 7-day culture derived from a well-differentiated adenocarcinoma cut tangentially to the attachment surface. The ectoplasm is filled with microfilaments, free ribosomes, some mitochondria, and microtubules. Microvilli extend from the cell surface. × 10,000.
Figs. 6 and 7. Electron micrographs of sections deeper in the cytoplasm in the cells seen in Fig. 5. All free surfaces of the cells bear microvilli. Autophagic vacuoles (A) and free ribosomes are abundant. Dense regions are seen beneath the plasma membrane at some points of the cell contacts (arrowheads). × 10,000.
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