

## **Mesenchyme-epithelial interactions in human endometrium. Prostaglandin synthesis in separated cell types.**

D Gal, ... , J M Johnston, P C MacDonald

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### **Research Article**

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# Mesenchyme-Epithelial Interactions in Human Endometrium

## PROSTAGLANDIN SYNTHESIS IN SEPARATED CELL TYPES

DAVID GAL, M. LINETTE CASEY, JOHN M. JOHNSTON, and PAUL C. MACDONALD,  
*Cecil H. and Ida Green Center for Reproductive Biology Sciences and the  
Departments of Biochemistry and Obstetrics-Gynecology, The University of  
Texas, Southwestern Medical School, Dallas, Texas 75235*

**ABSTRACT** Glandular epithelium and stromal cells of human endometrium were separated and maintained in monolayer culture. At the time the cells became confluent, cell suspensions were prepared and incubated with [ $^{14}\text{C}$ ]arachidonic acid. Radiolabeled prostaglandin  $\text{E}_2$  and, to a lesser extent, prostaglandin  $\text{F}_{2\alpha}$  and metabolites of these prostaglandins, were formed principally in stromal cells. There was considerably less prostaglandin formation in endometrial glands either after maintenance in monolayer culture or in freshly separated glands. In stromal cells of endometrium prostaglandin formation was linear with time of incubation for 2.5 min and with [ $^{14}\text{C}$ ]arachidonic acid concentrations up to 8  $\mu\text{M}$ . When stromal cells and epithelial cells were combined, all prostaglandin formation could be accounted for by that produced in stromal cells. Little or no prostaglandin formation was detected in stromal cells from human adipose tissue or in fibroblasts from human genital or abdominal skin or human fallopian tube.

## INTRODUCTION

Based on the findings of the elegant studies of a number of investigators (1-7), it is now clear that the differentiation and function of endothelial and epithelial cells may be regulated by interactions with mesenchyme or stroma (1). For example, it has been shown that androgen-induced differentiation of the urogenital sinus epithelium is dependent upon mesenchyme (2). Differentiation and development of embryonic endoderm or ectoderm of pancreas (3), liver (4), lung (5, 6), and thyroid (7) were shown to be dependent

upon mesenchymal factors. The same is true of the müllerian duct. Indeed it has been demonstrated that certain segments of mesenchyme from the müllerian duct direct differentiation into fallopian tube whereas other segments direct epithelial differentiation to uterus, uterine cervix, or vagina (2).

Generally it has been shown that normally functioning mesenchyme serves to deter malignant transformation of contiguous epithelial cells (8). For example, embryonic mammary mesenchyme will bring about differentiation of breast cancer cells (9) and basal cell carcinomas differentiate when transplanted to the uterus (1). Thus, stroma-mediated regulation of epithelial cell function is an established biological phenomenon.

The human endometrium is characterized by a well defined epithelial component (endometrial glands) and stroma, each of which appears to be hormonally responsive. Indeed, it is the hormone-induced alteration of the endometrial stromal cell that gives rise to the decidual cell characteristic of the decidua of human pregnancy. If the glandular epithelium of human endometrium was influenced by metabolic events that take place in contiguous stromal cells, as is the case in prostate tissue (2), a definition of such interaction may be essential to an understanding of the physiology as well as the pathophysiology of this tissue.

It is believed that prostaglandins (PG)<sup>1</sup> serve important functions in the physiology and possibly the pathophysiology of human endometrium. It seems likely that PG are important in the initiation of menstruation (10) and that PG synthesized in endometrium

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<sup>1</sup> Abbreviations used in this paper: PG, prostaglandin(s); Ab/Am, antibiotic-antimycotic mixture; TLC, thin-layer chromatography.

may cause the dysmenorrhea of progesterone withdrawal menses (11). Moreover, it has been proposed that PG synthesized in decidua lead to the initiation of parturition (12).

For these reasons, the purpose of the present investigation was to evaluate the pattern and rate of PG biosynthesis in separated glandular epithelium and stromal cells of human endometrium.

## METHODS

**Establishment of cell cultures.** Endometrial tissues were obtained directly from the uterus of premenopausal women immediately after hysterectomy conducted for reasons other than endometrial disease. The day of the ovarian cycle at the time of hysterectomy was established from menstrual history, histologic features of the endometrium, and the concentration of estradiol-17 $\beta$  and progesterone in serum on the day before surgery. Steroids were quantified by radioimmunoassay (13, 14).

Endometrial glands were separated from the stromal cells according to Satyaswaroop et al. (15) method. Briefly, the endometrial tissue was removed from the uterus under aseptic conditions in a laminar flow tissue culture hood. After the removal of blood clots, the tissue was placed in Hanks' balanced salt solution (HBSS) (Gibco Laboratories, Grand Island Biological Co., Grand Island, NY) that contained an antibiotic-antimycotic (Ab/Am) mixture (10%, vol/vol) (Gibco Laboratories). The mixture consisted of penicillin, 10,000 U/ml, fungizone, 25  $\mu$ g/ml, and streptomycin, 10,000  $\mu$ g/ml. After 30 min the tissue was transferred into HBSS that contained collagenase (1 mg  $\cdot$  ml<sup>-1</sup>; 196 U  $\cdot$  mg<sup>-1</sup>) (Millipore Corp., Freehold, NJ), deoxyribonuclease I (0.5 mg  $\cdot$  ml<sup>-1</sup>; 1,950 K U  $\cdot$  mg<sup>-1</sup>) (Sigma Chemical Co., St. Louis, MO), Hepes (25 mM) buffer, and Ab/Am (2%, vol/vol). The endometrium was cut into pieces ( $\sim$ 1 mm<sup>3</sup>) and then transferred in the collagenase-containing medium into a sterile tube and incubated at 37°C in a shaking water bath for  $\sim$ 1 h. The collagenase-treated tissue was strained through a stainless steel sieve with a pore size of 75- $\mu$ m (Millipore Corp.). The endometrial glands were retained by the sieve whereas the stromal cells passed through the sieve into the filtrate. The glands were washed with HBSS containing Ab/Am (2%) and placed in HAM F-10 culture medium (Gibco Laboratories) that contained fetal calf serum (10%) (Gibco Laboratories), insulin (10 mg  $\cdot$  ml<sup>-1</sup>), glucose (5 mg  $\cdot$  ml<sup>-1</sup>), and Ab/Am (1%). After 30 min at 37°C, during which time contaminating stromal cells attached to the dishes, the glands were transferred into 100-mm petri dishes that contained the same culture medium.

The stromal cells in the filtrate were washed two times with Waymouth's MB 752/1 culture medium (Gibco Laboratories) that contained heat-inactivated fetal calf serum (15%) and Ab/Am (1%). The stromal cells were divided among several petri dishes. The culture media were changed every 24 h. The cells were harvested for study at the time that confluency was attained, after  $\sim$ 10 d in the case of stromal cells and 2–3 wk in the case of epithelial cells.

Stromal fibroblasts derived by the same method from human fallopian tubes were prepared and maintained in culture. Fibroblasts derived from human genital and abdominal skin (16) were kindly provided by Dr. J. E. Griffin, The University of Texas Southwestern Medical School, Dallas, TX. Adipose tissue stromal cells (17) were kindly provided by Dr. E. R. Simpson, The University of Texas Southwestern Medical School.

**Assay of PG biosynthesis.** 4 h before assay of PG syn-

thesis, the culture medium was changed to one that contained no serum. Thereafter, the cells were removed from the dishes after trypsin (Gibco Laboratories) treatment and washed once with medium containing trypsin inhibitor (Sigma Chemical Co.) and serum and then once with medium that contained no serum or trypsin inhibitor.

The substrate used for this assay, [<sup>14</sup>C]arachidonic acid (Amersham Corp., Arlington Heights, IL), was purified before use by chromatography on a Unisil (Clarkson Chemical Co., Williamsport, PA) column. The chloroform washings of this column contained purified [<sup>14</sup>C]arachidonic acid; the chloroform was evaporated and the residue was suspended in ethanol and diluted with culture medium (Waymouth MB 752/1) that contained no serum. The final solution contained ethanol (1%) and [<sup>14</sup>C]arachidonic acid ( $0.23 \times 10^6$  dpm  $\cdot$   $\mu$ g<sup>-1</sup>).

The washed cells were suspended in medium that contained no serum and 1 ml of the cell suspension was transferred to tubes that were placed in a water bath at 37°C. The viability of both stromal and glandular epithelial cells was  $>90\%$  as judged by trypan blue exclusion. Other aliquots were taken for cell protein measurement that was conducted according to the method of Lowry et al. (18). The reaction was initiated by addition of [<sup>14</sup>C]arachidonic acid (in 1 ml of medium) to the cell suspension. The tubes were maintained at 37°C in a shaking water bath throughout the incubation period. The reaction was terminated by the addition of acetic acid (500  $\mu$ l) to the incubation mixture and placement of the tubes in ice. The incubation mixtures were treated by sonication to disrupt the cells and the lipids were extracted with 10 ml of chloroform/methanol (2:1, vol/vol). After centrifugation, to partition the solvents, the aqueous phase was removed and the chloroform phase was evaporated under a stream of N<sub>2</sub>. The residue was dissolved in methanol (20  $\mu$ l) and chloroform (500  $\mu$ l) and applied to a 25-mm column containing 500 mg Unisil. The columns were washed with chloroform to remove most of the nonmetabolized [<sup>14</sup>C]arachidonic acid. The PG were eluted from the column with chloroform/methanol (10:1, vol/vol). The eluate was evaporated and the PG were separated by thin-layer chromatography (TLC) on Polygram Sil G/HY silica gel (Brinkmann Instruments, Inc., Westbury, NY) using a solvent system of chloroform/methanol/acetic acid/water (90:8:1:0.8, by volume). Chromatography solvents were prepared fresh daily; chromatography tanks were lined with filter paper and were filled with N<sub>2</sub> and equilibrated before use. The areas on the chromatogram containing radioactive substances were localized by use of a Berthold thin-layer radiochromatogram scanner. Authentic samples of nonradiolabeled PGE<sub>2</sub>, PGF<sub>2 $\alpha$</sub> , thromboxane B<sub>2</sub>, 13,14-dihydro-15-keto-PGF<sub>2 $\alpha$</sub>  (Upjohn Co., Kalamazoo, MI), and 15-keto-PGF<sub>2 $\alpha$</sub> , 15-keto-PGE<sub>2</sub>, and 13,14-dihydro-15-keto-PGE<sub>2</sub> (provided as a generous gift by Dr. J. E. Pike, Upjohn Co.) were chromatographed in lanes parallel to those of the assay samples. These compounds were localized on the chromatograms after exposure to iodine vapor. After evaporation of the iodine the areas of the chromatograms of the assay samples corresponding to the R<sub>f</sub> values of the known standards were removed, placed in scintillation vials containing methanol (10%) in OCS (Amersham Corp.), and assayed for radioactivity in a liquid scintillation spectrometer. All experimental values were corrected for blank values. Incubations without cells were conducted and the amounts of radioactivity from these incubations which migrated on the chromatograms with a similar R<sub>f</sub> to that of the products, was subtracted from the experimental values. The recovery of PG products was estimated from the recovery of [<sup>3</sup>H]PGE<sub>2</sub>, [<sup>3</sup>H]PGF<sub>2 $\alpha$</sub> , [<sup>3</sup>H]13,14-dihydro-15-keto-PGE<sub>2</sub>, and [<sup>3</sup>H]13,14-

dihydro-15-keto-PGF<sub>2α</sub> (Amersham Corp.) in reaction mixtures that contained no cells. Recoveries for these PG products were ~65, 55, 78, and 68%, respectively.

## RESULTS

14 endometrial specimens were processed for cell culture. Glandular epithelial cells in monolayer culture were established successfully in 8 of the 14. In most instances the failure to establish glandular epithelial cells in culture was due to infection. Stromal cells in monolayer culture were established from 12 of the 14 endometrial samples. Contamination of stromal cell cultures or cultures of glandular epithelium by cells of the opposite type was judged to be <5% by repeated microscopic examinations throughout the culture period. The microscopic appearance of glandular epithelium and stromal fibroblasts in monolayer culture was quite different (Fig. 1). To evaluate the viability of cells after dispersion by collagenase treatment, an aliquot of the cell suspension from both glands and stromal cells was stained with trypan blue. Based on the results of the dye exclusion test (19), 95% of the

glandular epithelial cells were viable whereas only 15% of the stromal cells were viable immediately after collagenase treatment.

*PG formation in stromal cells and in glandular epithelium of human endometrium.* Initially, experiments were conducted to compare the formation of [<sup>14</sup>C]PG from [<sup>14</sup>C]arachidonic acid by stromal cell fibroblasts and glandular epithelial cells in suspension. These cells were incubated separately or in combination (1:1). Each incubation mixture contained the same amount of total cell protein. The cells were incubated for 60 s at 37°C in the presence of [<sup>14</sup>C]arachidonic acid (9 μM). In these studies we found that glandular epithelial cells synthesized [<sup>14</sup>C]PG from [<sup>14</sup>C]arachidonic acid to a much lesser degree than the stromal cells prepared from the same endometrium. The [<sup>14</sup>C]PG formed in cultures that contained a 1:1 mixture of glandular epithelial cells and stromal fibroblasts could be attributed almost entirely to [<sup>14</sup>C]PG formed by stromal cells since [<sup>14</sup>C]PG formed was proportional to the cell protein of stromal fibroblasts present in these cultures (Fig. 2).

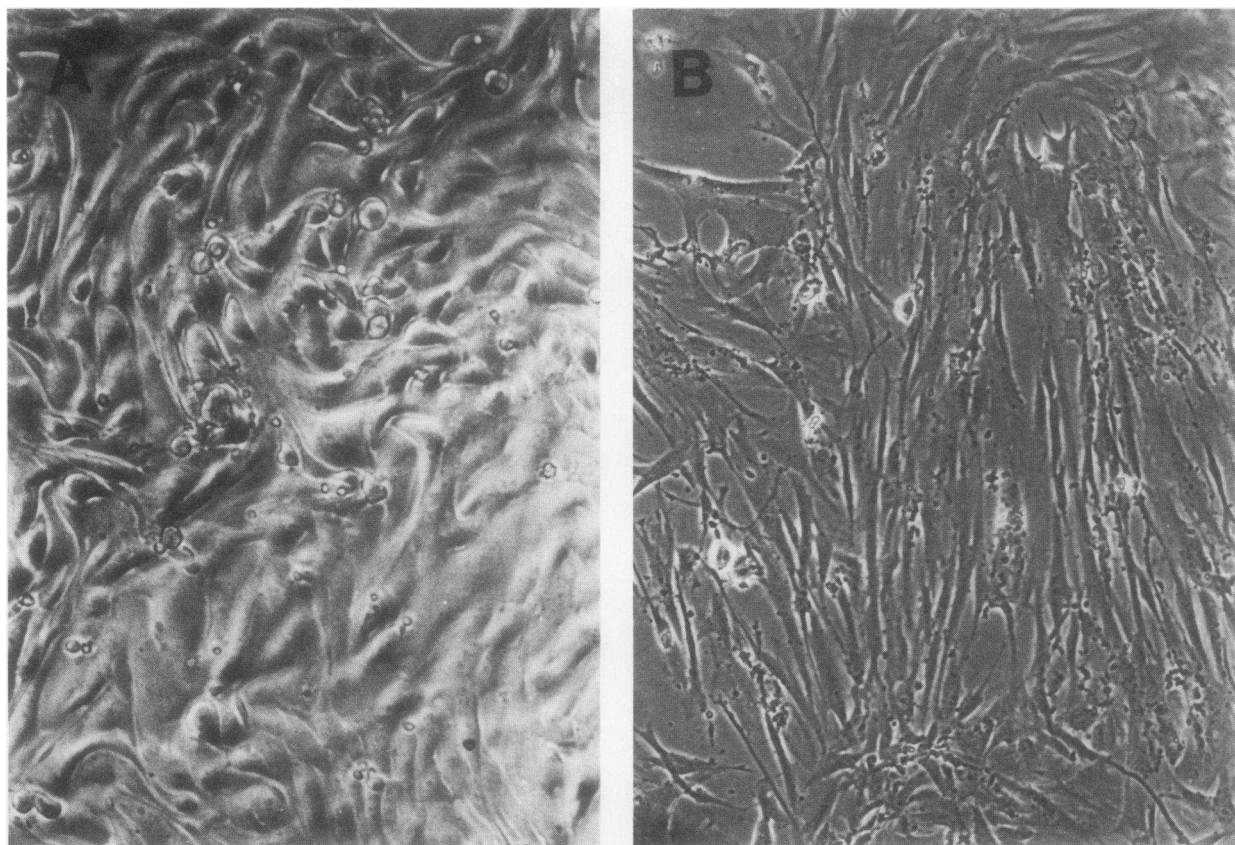


FIGURE 1 Endometrial glandular epithelial cells (A) and endometrial stromal fibroblasts (B) in monolayer cultures,  $\times 200$ .

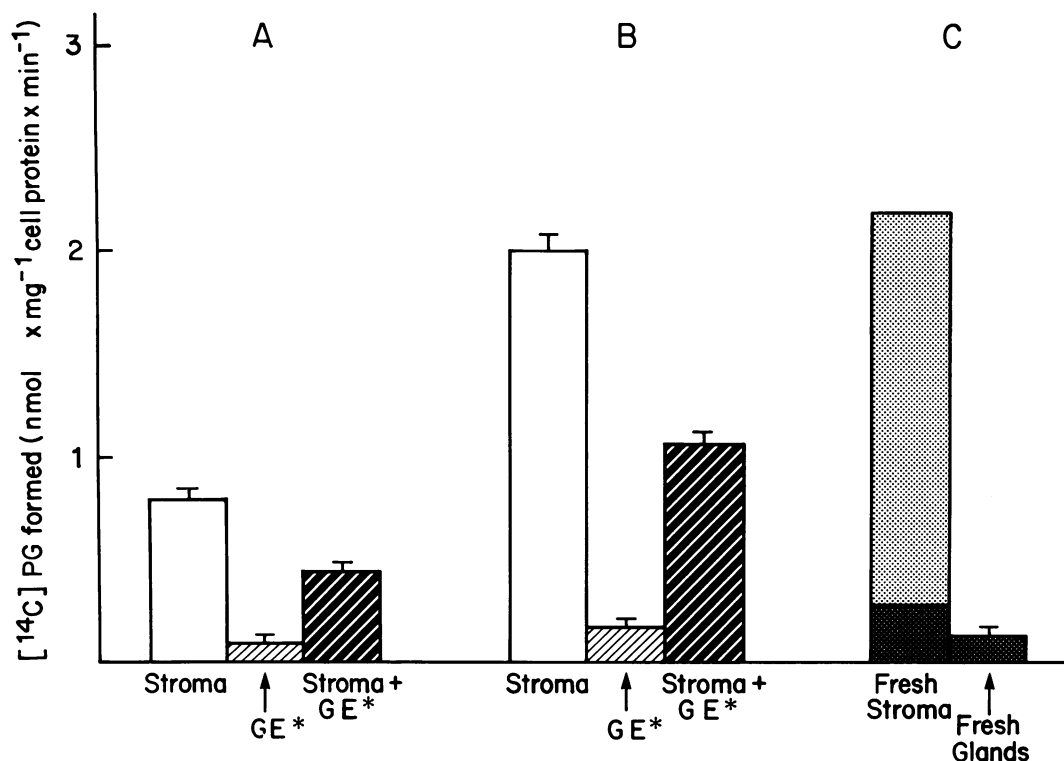


FIGURE 2 Production rate (mean $\pm$ SD) of [ $^{14}$ C]PG of the (A) F group (PGF $_{2\alpha}$  + 15-keto-PGF $_{2\alpha}$  + 13,14-dihydro-15-keto-PGF $_{2\alpha}$ ) and (B) the E group (PGE $_2$  + 15-keto-PGE $_2$  + 13,14-dihydro-15-keto-PGE $_2$ ) in stromal cells (stroma), glandular epithelial cells (GE\*), and a mixture (1:1) of both (stroma + GE\*) after incubation with [ $^{14}$ C]arachidonic acid (9  $\mu$ M) for 60 s at 37°C. (C) Production of [ $^{14}$ C]PG in freshly prepared glands and stromal cells after incubation with [ $^{14}$ C]arachidonic acid (9  $\mu$ M) for 60 s at 37°C. The dark areas represent the actual [ $^{14}$ C]PG formed  $\cdot$  mg $^{-1}$  cell protein in each mixture and the total height of the bar represents nanomoles PG formed  $\cdot$  mg $^{-1}$  cell protein corrected for living cells—as estimated by the dye exclusion test (Methods). Experiments were conducted with cells from eight different endometrial tissues.

To address the question as to whether the capacity to form [ $^{14}$ C]PG by the glandular epithelial cells may have been lost during time in culture, freshly separated epithelial glands and stromal cells were incubated with [ $^{14}$ C]arachidonic acid (9  $\mu$ M) at 37°C for 60 s and assayed for [ $^{14}$ C]PG formation. In these studies we found that fresh glands, as in the case of glandular epithelial cells that had been maintained in monolayer culture, did not form appreciable amounts of [ $^{14}$ C]PG (Fig. 2). [ $^{14}$ C]PG were not formed when indomethacin (1.7  $\mu$ M) was added to the medium of stromal cells before the addition of [ $^{14}$ C]arachidonic acid. Also there were no [ $^{14}$ C]PG formed when cells were absent (Fig. 3).

*PG formation in fibroblasts derived from endometrial stroma, adipose stroma, genital skin, nongenital skin, and endometrial glandular epithelium.* The rate of PG formation from [ $^{14}$ C]arachidonic acid in fibroblasts derived from various tissues and endometrial glandular epithelium was compared with that in

endometrial stromal fibroblasts. We found that the endometrial stromal cells formed PG at a relatively high rate ( $\sim 3$  nmol PG  $\cdot$  min $^{-1}$   $\cdot$  mg $^{-1}$  protein) whereas the fibroblasts derived from other tissues and endometrial glandular epithelial cells did not synthesize PG efficiently (Fig. 4).

*Radiolabeled PG formation from [ $^{14}$ C]arachidonic acid by endometrial stromal cells as a function of incubation time and substrate concentration.* The formation of radiolabeled PG from [ $^{14}$ C]arachidonic acid in endometrial stromal cells was evaluated as a function of time of incubation. The results of these studies, conducted in six separate samples of cells, showed that [ $^{14}$ C]PG were formed in a linear fashion as a function of time up to 2.5 min. When time of incubation was  $>2.5$  min, product formation was no longer linear with time of incubation (Fig. 5). No more than 12% of the [ $^{14}$ C]arachidonic acid added as substrate was depleted by total [ $^{14}$ C]PG production during

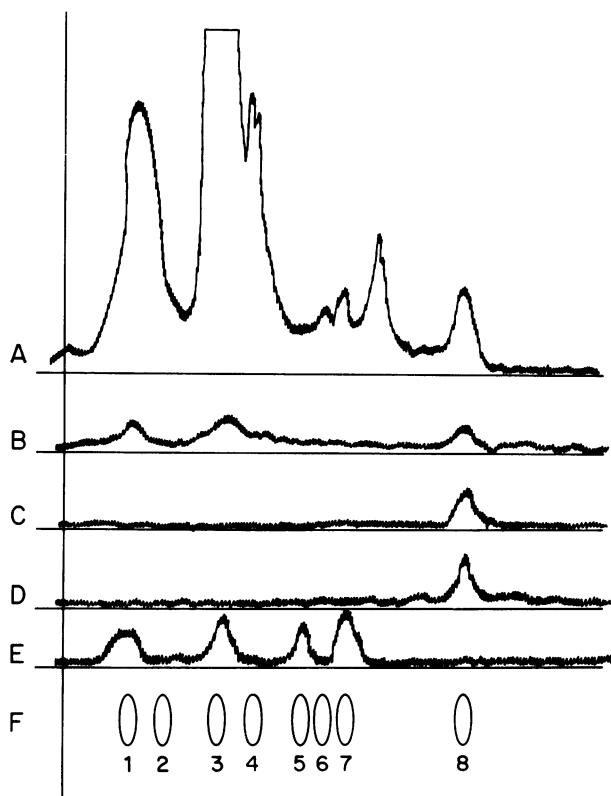


FIGURE 3 Radiochromatogram of [ $^{14}\text{C}$ ]PG products formed in endometrial stromal cells (A), glandular epithelial cells (B), endometrial stromal cells treated with indomethacin ( $1.6\ \mu\text{M}$ ) (C), medium containing no cells that served as blank incubated with [ $^{14}\text{C}$ ]arachidonic acid ( $9\ \mu\text{M}$ ) for 60 s at  $37^\circ\text{C}$  (D). The recovery of [ $^3\text{H}$ ]PGF $_{2\alpha}$ , [ $^3\text{H}$ ]PGE $_2$ , [ $^3\text{H}$ ]13,14-dihydro-15-keto-PGF $_{2\alpha}$ , and [ $^3\text{H}$ ]13,14-dihydro-15-keto-PGE $_2$ , which were added to medium with no cells is indicated in lane E. The following nonradiolabeled products were used as chromatography standards (F): (1) PGF $_{2\alpha}$ ; (2) thromboxane B $_2$ ; (3) PGE $_2$ ; (4) 15-keto-PGF $_{2\alpha}$ ; (5) 13,14-dihydro-15-keto-PGF $_{2\alpha}$ ; (6) 15-keto-PGE $_2$ ; (7) 13,14-dihydro-15-keto-PGE $_2$ ; (8) arachidonic acid.

an incubation period of 60 s. Therefore, we chose to limit the incubation time to 60 s in subsequent experiments. It is also apparent from the results of these experiments that stromal cells formed considerably more [ $^{14}\text{C}$ ]PGE $_2$  products than [ $^{14}\text{C}$ ]PGF $_{2\alpha}$  products from [ $^{14}\text{C}$ ]arachidonic acid and only trace amounts of [ $^{14}\text{C}$ ]thromboxane B $_2$ , under these assay conditions.

To evaluate the effect of the concentration of [ $^{14}\text{C}$ ]arachidonic acid on the formation of [ $^{14}\text{C}$ ]PG by stromal cells in suspension, [ $^{14}\text{C}$ ]arachidonic acid, in various amounts, was added to suspensions of stromal cells that were then maintained at  $37^\circ\text{C}$  for 60 s. In experiments with four separate samples of stromal cells, we found that [ $^{14}\text{C}$ ]PG were produced in a concentration-dependent fashion up to a concentration of

$8.5\ \mu\text{M}$  [ $^{14}\text{C}$ ]arachidonic acid, above which there was no further significant increase in product formation (Fig. 6). We found no differences in the production of [ $^{14}\text{C}$ ]PG (or metabolites thereof) from exogenously added [ $^{14}\text{C}$ ]arachidonic acid among the various endometrial stromal cells tested.

Since 6-keto-PGF $_{1\alpha}$  and PGF $_{2\alpha}$  comigrate in the solvent system used for TLC, we conducted additional experiments to evaluate the production of prostacyclin (measured as 6-keto-PGF $_{1\alpha}$ ) by endometrial stromal and glandular epithelial cells. In these studies, we used two solvent systems [chloroform/methanol/acetic acid, 98:2:5 (by volume) and diethyl ether/methanol/acetic acid, 90:1:2 (by volume)] in which 6-keto-PGF $_{1\alpha}$  and PGF $_{2\alpha}$  have different mobilities but PGE $_2$  and 15-keto-PGF $_{2\alpha}$  comigrate. We found that very low or undetectable amounts of [ $^{14}\text{C}$ ]prostacyclin (measured as [ $^{14}\text{C}$ ]6-keto-PGF $_{1\alpha}$ ) were produced by either endometrial stromal or glandular epithelial cells.

## DISCUSSION

The regulation of PG synthesis and inactivation in endometrium is believed to be of signal importance in the initiation of menstruation and of parturition (10, 12). Moreover, these processes appear to be, either directly or indirectly, controlled by the hormonal milieu of the ovarian cycle (11, 20) or of pregnancy (21). It has been demonstrated by others (22, 23) that estrogen stimulates PG synthesis and/or release in uterine tissues. Thus, the question arose as to the cellular site of PG synthesis in endometrial and decidual tissues. To define these metabolic events more precisely, this investigation was undertaken to evaluate the capacity for, as well as the nature of, PG biosynthesis in the cell types of human endometrium. We used separated glandular epithelial cells and stromal cells maintained before assay in monolayer culture. There was considerable PG formation, principally PGE $_2$ , but also PGF $_{2\alpha}$ , in the stromal cells but not in the glandular epithelial cells of endometrium from exogenous [ $^{14}\text{C}$ ]arachidonic acid. These findings could not be explained by a loss of prostaglandin synthase activity in glandular cells during time in culture since little PG was formed from [ $^{14}\text{C}$ ]arachidonic acid in freshly prepared glands. Moreover, the endometrial stromal cells produced large amounts of radiolabeled PG from [ $^{14}\text{C}$ ]arachidonic acid compared with that produced in fibroblasts from genital and nongenital skin, adipose tissue, and fallopian tube.

We are mindful of the possibility that the nature and rate of PG synthesis from exogenous arachidonic acid may differ considerably from that derived from endogenous precursor released from esterified stores. Indeed, this has been shown to be the case in cultured

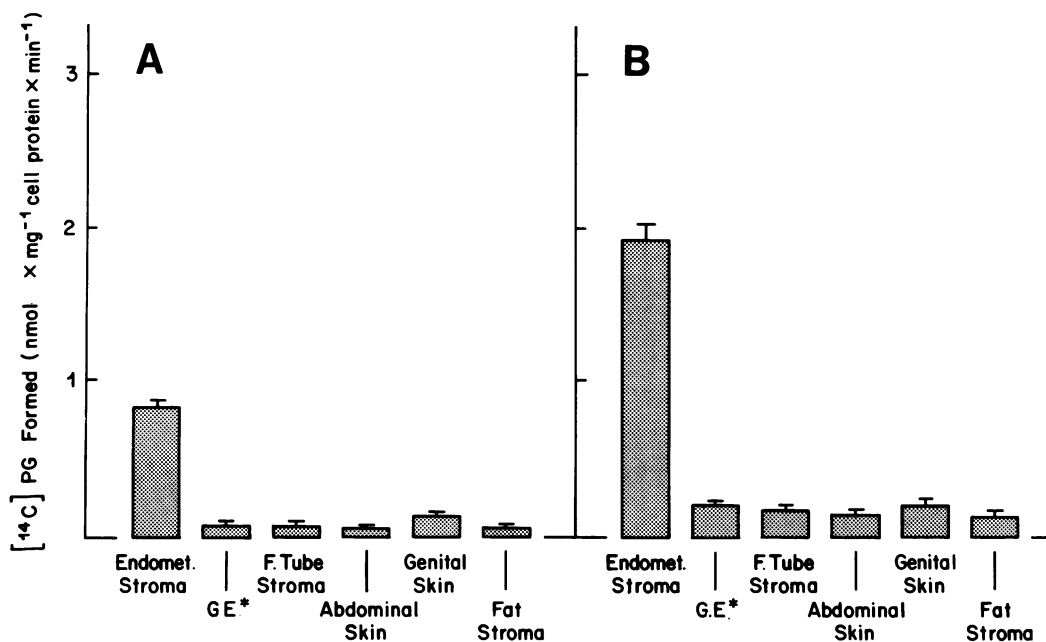


FIGURE 4 Production rate (mean $\pm$ SD) of  $[^{14}\text{C}]$ PG of the F group (A) and the E group (B) by various cells in monolayer culture after incubation with  $[^{14}\text{C}]$ arachidonic acid ( $9\text{ }\mu\text{M}$ ) for 60 s at  $37^\circ\text{C}$ . G.E\*, endometrial glandular epithelium; F. tube, fallopian tube. Experiments were conducted in duplicate.

rabbit mesothelial cells (24) and in rabbit kidney medullary tissue (25). Nonetheless, there was a striking difference in the capacity of endometrial stromal cells

and endometrial epithelium and fibroblasts derived from several tissues to synthesize radiolabeled prostanooids from  $[^{14}\text{C}]$ arachidonic acid. Thus, the capacity

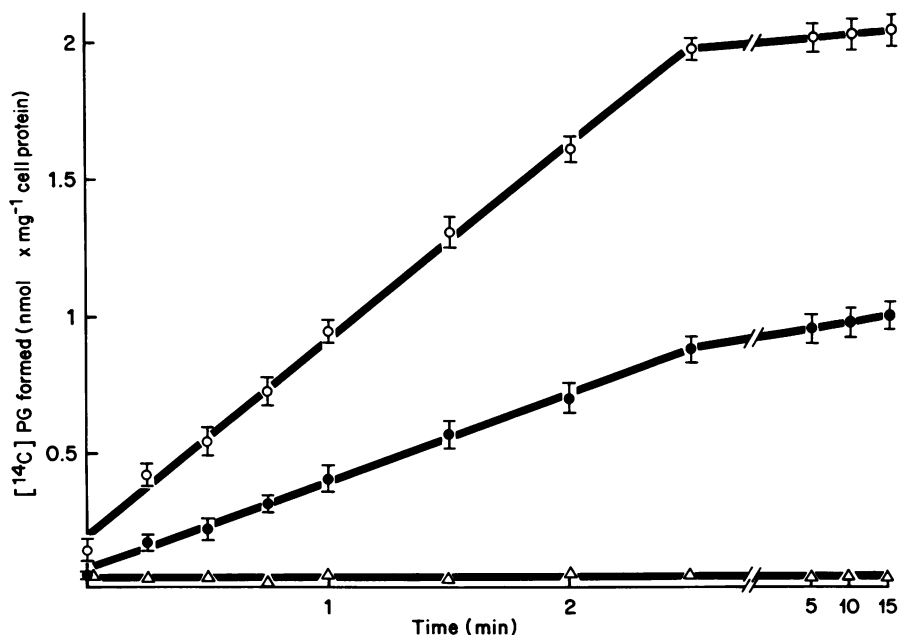


FIGURE 5 Production rate (mean $\pm$ SD) of radiolabeled PGE $_2$  + 15-keto-PGE $_2$  + 13,14-dihydro-15-keto-PGF $_{2\alpha}$  + 15-keto-PGF $_{2\alpha}$  + 13,14-dihydro-15-keto-PGF $_{2\alpha}$  (O); and thromboxane (Δ), by endometrial stromal cells incubated with  $[^{14}\text{C}]$ arachidonic acid ( $9\text{ }\mu\text{M}$ ) at  $37^\circ\text{C}$  as a function of incubation time. Experiments were conducted in duplicate.

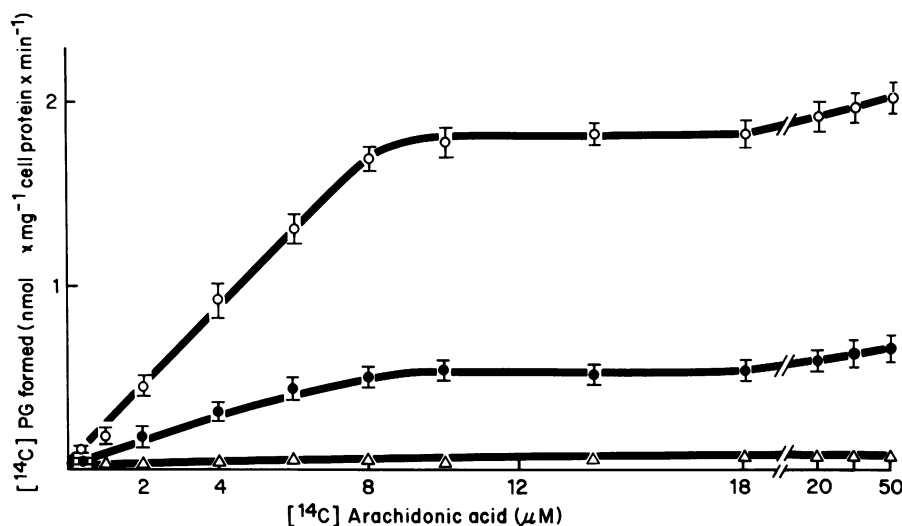


FIGURE 6 Production rate of radiolabeled PGE<sub>2</sub> + 15-keto-PGE<sub>2</sub> + 13,14-dihydro-15-keto-PGE<sub>2</sub> (O); PGF<sub>2α</sub> + 15-keto-PGF<sub>2α</sub> + 13,14-dihydro-15-keto-PGF<sub>2α</sub> (●); and thromboxane (Δ) by endometrial stromal cells as a function of concentration of [<sup>14</sup>C] arachidonic acid. Experiments were conducted in duplicate.

to form PG in large amounts is not a characteristic of all mesenchymal fibroblasts.

In a previous investigation we found that NAD<sup>+</sup>-dependent 15-hydroxyprostaglandin dehydrogenase activity in human endometrium was localized principally in the glandular epithelium (26). Moreover, the specific activity of the enzyme varied greatly in endometrium during the ovarian cycle, being greatest during the midluteal phase. These findings are suggestive that the activity of the enzyme is stimulated by progesterone (26). These findings, together with those of the present study, also are suggestive of a unique relationship between glandular epithelium and stroma of endometrium; PG is formed from exogenous arachidonic acid primarily in stroma but is inactivated primarily in the glandular epithelium. With respect to the utilization of exogenous arachidonic acid for PG formation, the decidua vera of the uterus of pregnant women may be unique in this regard. It has been demonstrated that large amounts of arachidonic acid are released from the avascular fetal membranes during human labor (27); the fetal membranes are contiguous to the decidualized stromal cells and, thus, may provide PG precursor to the decidua.

Based on the findings of the present investigation we conclude that PG biosynthesis in human endometrium is principally a function of the stromal cells, at least when exogenous substrate is available. On the other hand, PG inactivation is principally a function of the progesterone-stimulated glandular epithelium (26). We speculate that estrogen-induced PG synthesis

in stroma will lead to PG that affects the metabolic events in glandular epithelium in which the capacity for PG inactivation is reduced, i.e., when progesterone levels are low.

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