Isolation and Characterization of Rabbit Endocervical Cells

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ABSTRACT Three cytologically distinct cell populations were identified, in addition to ciliated cells, when a unit gravity sedimentation procedure was applied to pronase-dispersed rabbit endocervical cells. Two of these cell populations contained histochemically distinguishable (periodic acid-Schiff [PAS]) mucoproteins and were designated vacuolated and granular PAS-positive cells. The third, designated as vacuolated PAS-negative, did not contain secretory granules. Cell integrity was confirmed by trypan blue dye exclusion, [3H]leucine incorporation, and ultrastructural analysis. To demonstrate hormonal modulation of endocervical cell morphology, cell distribution profiles were compared from animals in different hormonal states. In the absence of estrogen dominance, PAS-positive cells from 5-d pseudopregnant rabbits were reduced 50%, while vacuolated PAS-negative cells increased fourfold as compared with estrous cell populations. The PAS-positive cells sedimented toward the top of the gradient where the bovine serum albumin concentrations were lower, consistent with a reduction in the number of secretory granules. In the sustained absence of ovarian steroid hormones, the number of PAS-positive mucous cells from ovariectomized rabbits was reduced to only 4% of the total endocervical cell population.

The biosynthetic capacity of isolated endocervical cells was determined by incubating the three nonciliated cell populations from estrous and 5-d pseudopregnant rabbits for 36 h with the mucin precursor, [14C]N-acetyl-d-glucosamine. Only PAS-positive cells incorporated significant amounts of the radiolabel. Specific biosynthetic and secretory activities were the same for vacuolated and granular PAS-positive cells from both estrous and pseudopregnant rabbits. Cells isolated from ovariectomized rabbits did not incorporate significant amounts of labeled precursor. This study indicates that steroid hormones influence cervical secretions by modulating the type of endocervical cells.

Steroid hormone modulation of gene expression and cytodifferentiation in reproductive tract tissues is well documented for the oviduct, endometrium, and ectocervix in several species (4, 46). Similar documentation is unavailable for the endocervix, despite the recognition of hormone-regulated cyclic changes in the physicochemical characteristics of cervical mucus (1, 16, 31, 44).

In the human cervix, a steroid target organ, it is generally accepted that estrogen stimulates the production of a thin, watery mucus, while progesterone dominance results in a dry, viscous mucus (26, 49). Hormone-induced variations in the biophysical properties of cervical mucus regulate sperm and bacterial access to the upper reproductive tract of cycling (human) as well as noncycling (rabbit) animals (2, 6, 27). Although it is generally acknowledged that steroid hormones act directly on target tissues, it is possible that changes in the mucus are enacted outside of endocervical cells. Possibilities include alterations in mucus volume caused by transudation or in mucus composition caused by the contribution of blood, locally produced proteins, and other reproductive tract secretions (3, 9, 48). Alternatively, endocervical mucous cells, as cells of other mucous epithelia, could be responsible for the production of secretions with different physicochemical characteristics (21). In support of the latter hypothesis are the studies of Odor (32) and Riches et al. (41) in the rabbit endocervix. These authors noted a decrease in the number and type of secretory granules in endocervical cells after ovariectomy and their restoration after the administration of estrogen.
They also indicated that the number, location, and structure of mucous granules were determined by the predominant hormonal status.

To assess the possibility that ovarian hormones modulate endocervical cell morphology, we have characterized endocervical cell populations from rabbits in different hormonal states after their isolation by unit gravity sedimentation. The results indicate that, in addition to ciliated cells, there are three cytologically distinct, nonciliated cell populations in the endocervix. These cells undergo marked changes in number and sedimentation properties as a function of the changing hormonal milieu.

**MATERIALS AND METHODS**

**Preparations of Single Cell Suspensions**

Mature, virgin, New Zealand White rabbits (6 mo or older) were housed individually for 3 wk to insure against reflex ovulation resulting in pseudopregnancy at the time of experimentation. Rabbits were either estrous, pseudopregnant, or ovariectomized. Pseudopregnancy was induced, 5 db before sacrifice, with an ear vein injection of 15-20 IU of human chorionic gonadotropin (Sigma Chemical Co., St. Louis, Mo.) followed by cervical stimulation. Ovariectomized animals were maintained for 15-16 wk before use. Rabbits were sacrificed with an ear vein injection of 70% ethanol, and cervixes were removed within 5 min of death. Cervical folds were stripped mechanically, washed in Hank's balanced salt solution (Grand Island Biological Co. [GIBCO], Grand Island, N. Y.) containing antibiotics (5). All cell 500 IU/ml streptomycin sulfate, 200 µg/ml, and mixed with scalp blades into 4-mm fragments.

Cell suspensions were prepared by incubating tissue fragments on an orbital shaker (70 rpm) in a solution composed of 0.5% pronase (45,000 proteolytic units/ml; 0.1% bovine serum albumin (BSA), fraction V, Sigma Chemical Co.) dissolved in Tissue Culture Medium 199 (TCM; GIBCO). Incubation was carried out at 37°C for 40 min under 5% CO2 in air. Dissociation was augmented by repeatedly pipetting tissue fragments through a small bore (1.5 mm outside diameter) Pasteur pipette. Subsequently, fragments were washed free of pronase and collected by centrifugation (1,500 rpm, 5 min). The resultant pellet was resuspended in calcium-magnesium-free TCM 199 containing 0.1% BSA, and cell dissociation was completed by repeated aspiration of tissue fragments through a fine bore (0.75 mm outside diameter) Pasteur pipette. Underdigested stromal fragments were removed by filtration through a 250-µm nylon mesh filter (Small Parts, Inc., Miami, Fla.). Dissociated cells were collected by centrifugation and resuspended in 10 ml of 0.1% BSA in TCM 199 in preparation for separation by unit gravity sedimentation. Viability was evaluated by the trypan blue dye exclusion test (25).

**Cell Separation and Morphology**

The chamber (500-ml capacity) and methods of Peterson and Evans (36) and Hymer et al. (18) for cell separation were employed with the following modifications: (a) BSA solutions prepared in TCM 199 and antibiotics (penicillin, 250 IU/ml; streptomycin sulfate, 100 µg/ml) were filtered through a Millipore Filling System equipped with 1.0-, 0.45-, and 0.22-µm filters (Millipore Corp., Bedford, Mass.); (b) cell suspensions were applied to the top of the gradient with a cannula attached to a 10-ml syringe; (c) only 1.6-2.0 x 106 cells suspended in 0.1% BSA were separated on the gradient; (d) a settling time of 1.5 h was employed. 36 fractions (15 ml each) were collected by pumping the gradient through the top of the chamber. Cells were concentrated by centrifugation (2,000 rpm, 5 min), resuspended in 0.5 ml of TCM 199, and counted with a hemocytometer. For histological studies, an aliquot of each fraction (1.2 x 106 cells) was pelleted (1,400 rpm, 10 min) onto a microscope slide in a Shandon cytocentrifuge (Shandon Southern Instruments, Inc., Sewickley, Pa.).

Cells were fixed with formalin-acetic acid-alcohol and stained for the presence of glycoproteins by a modified periodic acid-Schiff (PAS) technique (25) or fixed with methanol and stained for general morphological evaluation with Jenner-Giemsa. All preparations were pretreated with 0.1% diastase (0.16 M phosphate buffer, pH 6.0) for 30 min at room temperature to remove glycogen before staining. A red-magenta, PAS-positive reaction was taken as indicative of intracellular glycoproteins. After staining, the specific cell types were recorded by counting 500 cells within five preselected regions of each slide with the aid of a Whipple ocular crossline disc (Arthur H. Thomas Co., Philadelphia, Pa.). Enrichment was determined by comparing the percentage of each cell type before separation to that after separation, exclusive of erythrocytes.

For transmission electron microscopy, cells were fixed in 0.1 M cacodylate-buffered (pH 7.35) 3% glutaraldehyde at 4°C for 90 min; they were postfixed with 1% osmium tetroxide in the same buffer, dehydrated, and embedded in Epon 812 (24). To minimize cell loss caused by sample transfer, all steps were carried out in conical polyethylene centrifuge tubes (Bel-Art Products, Pequannock, N.J.). Thin (600-800 Å) sections were stained with uranyl acetate and lead citrate (40) and viewed with a Hitachi-12A electron microscope operated at 75 kV.

**Biosynthetic Activity of Isolated Cells**

Heterogeneous and enriched cell populations from estrous, pseudopregnant, and ovariectomized were collected by centrifugation and resuspended in 2.5 ml of D,L-leucine-free TCM 199 containing the following components: 0.1% BSA, 50 µg/ml gentamicin sulfate (Garamycin, Shering Pharmaceutical Corp., Kenilworth, N. J.), 2.5 µg/ml fungizone (E. R. Squibb & Sons, Princeton, N. J.). 2 µCi/ml [3H]leucine (New England Nuclear, Inc., Boston, Mass.; sp act, 60 Ci/mmol), 1 µCi/ml [14C]-N-acetyl-D-glucosamine [14C]NAG, New England Nuclear; sp act, 35.6 mCi/mmol). Cells were incubated for 36 h at 37°C under a 5% CO2-95% air atmosphere. Previous experiments indicated that similar preparations were capable of linear incorporation rates for both [3H]NAG and [3H]leucine over a 48-h period.

After incubation, cell suspensions were collected by centrifugation (2,000 rpm, 5 min). Supernatant solutions were saved, cells were resuspended in 0.5 ml of TCM 199, and sonicated for 20 min on ice. BSA (final concentration 4 mg/ml) was added as carrier protein to 0.25- and 0.5-ml aliquots of each cell sonicate and supernatant solution, respectively. Percihic acid (PCA) was then added to a final concentration of 0.2 N, and acid-precipitable material was recovered on borosilicate glass fiber filters (H. Reeve Angel & Co., Inc., Clifton, N. J., 934-1H). Filters were washed with 5-10 ml of ice-cold 0.2 N PCA until washes contained only background levels of radioactivity. Proteins were solubilized off filters in 1.0 ml of protocol (New England Nuclear) for 30 min at 60°C, and radioactivity was quantitated in an Intertechnique scintillation counter (INSUS Service Corp., Fairfield, N. J.) with counting efficiencies of 47 and 75% for 3H and 14C, respectively. Acid-precipitable radioactivity from the intracellular incorporation of [14C]NAG was taken as an index of mucin synthesis, while the presence of acid-precipitable radioactivity in the culture medium (supernatant solution) measured mucin secretion. Incorporation of [3H]leucine monitored cell viability. Student's t test was used for statistical evaluation of data.

**Fractionation of Radiolabeled Glycoproteins**

Cell sonicates (0.25 ml each) and the corresponding supernatant solutions (2.0 ml each) from BSA gradient fractions were pooled and dialyzed extensively against distilled water at 4°C to remove unincorporated radiolabeled precursors. Samples were lyophilized, subsequently solubilized in 0.2-0.3 ml of 0.22 M sodium thiocyanate-0.1 M Tris-HCl (pH 7.5) (22), and fractionated on Sepharose 2B columns (1.2 x 14.3 cm) with acidic elution buffer consisting of 0.01 M sodium thiosulfate-0.1 M Tris-HCl (pH 7.5), 2 B columns (1.2 x 14.3 cm), equilibrated with the same buffer. The protein content of the eluant was monitored by fluorescence spectrophotometry (excitation, 280 nm; emission, 340 nm). Radioactivity was quantitated in 1.0-ml aliquots of the column eluant.

**RESULTS**

**Characterization of Specific Cell Types**

As it is generally accepted that estrogens stimulate mucus production (26, 49), we initiated our studies with estrous rabbits, anticipating that endocervical cells derived from these animals would be maximally active in mucin synthesis and secretion. Pronase and mechanical disaggregation of endocervical folds yielded 1.7-2.7 x 106 single cells/cell, with a viability index of 95-98%, as measured by trypan blue dye exclusion. With rare exceptions, isolated cells showed no evidence of plasma membrane damage or leakage of secretory material. Before unit gravity separation, the cell suspension (Fig. 1) consisted of ~5% leukocytes, 6% vacuolated PAS-negative cells, 25% mucous cells (21% vacuolated and 79% granular PAS-positive cells), 6% ciliated cells, and 58% erythrocytes.

To 26 different experiments with estrous does, consistent enrichments were obtained after separation, and cell recoveries averaged 75-80% of the original inoculum. As shown in Fig. 2, gradients of 0.49-2.35% BSA were subdivided into 36 fractions
FIGURE 1  Low-magnification electron micrograph of dispersed endocervical cells before separation at unit gravity. Nonvacuolated (MC) and vacuolated (VC) mucous cells are present, along with a ciliated cell (CC) and two vacuolated cells that do not contain mucous granules (arrows). Note also numerous erythrocytes. Bar, 1 μm. X 4,500.

FIGURE 2  Characteristic distribution of endocervical cells from estrous rabbits separated on a continuous BSA gradient (empty circles). Cell number is expressed on a logarithmic scale and is plotted against gradient fraction number.

(15 ml each) with cell types banded from top to bottom in the sequence: erythrocytes, fractions 5–7; leukocytes, fractions 8–9; and four cell types identifiable within the epithelium of PAS-stained sections of intact endocervices, fractions 10–36.

Of the four "epithelial" cell types isolated, vacuolated PAS-negative cells had a lacy cytoplasm characterized by empty vacuoles as well as vacuoles containing traces of moderately electron-dense material and by dilated endoplasmic reticulum and filopodia (Fig. 3a). These cells ranged in size from 12 to 24 μm (diameter) and represented 86.5 ± 1.9% (mean ± SE) of the cells in fractions 10–14. Vacuolated PAS-positive cells, containing heterogeneous mucous granules and empty cytoplasmic vacuoles (11.5–17.2 μm diameter; Fig. 3b), constituted 60.1 ± 4.0% of the cells in fractions 15–21. Granular PAS-positive cells (Fig. 3c; 8.5–12.2 μm diameter) lacked vacuoles, had their cytoplasm engorged with heterogeneous mucous granules, and constituted 77.7 ± 3.2% of the cells in fractions 21–31. Approximately 30% of the cells localized in fractions 29–31 were ciliated (Fig. 3d; 14–20 μm diameter), and they displayed variable morphological features, including the presence or absence of vacuoles, intracellular lumina, and a few mucous granules. Ciliated cells were also found in fractions enriched for granular and, occasionally, for vacuolated PAS-positive cells.

In examining the possibility that the endocrine state of the animal modulates endocervical cell morphology, cells from progesterone-dominated (5-d pseudopregnant) does were also characterized. Before separation, these cell populations, 1.2–3.1 x 10⁶ cells/animal, contained 9% leukocytes, 26% vacuolated PAS-negative cells, only 12% mucous cells (8.5% vacuolated and 91.5% granular PAS-positive cells), 7% ciliated cells, and 46% erythrocytes. In seven different separation experiments (13 animals), cell recoveries were 73 ± 5% (mean ± SE) of the original inoculum.

Vacuolated PAS-negative cells were similarly distributed in gradients of pseudopregnant and estrous cell populations. This cell type represented 60–85% of the cells in fractions 10–13 (Fig. 4a), corresponding to enrichments of ~1.5- and 5.0-fold for estrous and pseudopregnant rabbits, respectively. The distribution of vacuolated PAS-positive cells showed marked
variation when estrous and pseudopregnant animals were compared (Fig. 4b). This cell type was enriched maximally in fractions 15–19 of estrous animal profiles (four- to fivefold), contrasted with fractions 13–16 for pseudopregnant animals (4–7 fold). Granular cells were also distributed differently in the gradients from estrous and pseudopregnant animals, with a shift toward the lower BSA concentrations apparent for the pseudopregnant rabbit (Fig. 4c). Thus, for pseudopregnant animals, granular cells predominated in fractions that were enriched for vacuolated PAS-positive cells in estrous animals.

Analysis of a distribution profile of cells derived from ovariectomized animals (n = 3) allowed an evaluation of cellular morphology in the absence of ovarian steroid influence. The initial cell population before separation was composed of 5% leukocytes, 23% vacuolated PAS-negative cells, 4% mucous cells (50% vacuolated and 50% granular PAS-positive cells), 6% ciliated cells, 37% erythrocytes, and 25% undefined PAS-negative cells. After separation, vacuolated PAS-negative cells from ovariectomized animals predominated throughout the entire gradient (Fig. 4a; 92% in fractions 5–9 and 35–67% in fractions 10–28), while a low percentage of both vacuolated and granular PAS-positive cells were scattered through gradient fractions 12–28 (Fig. 4b and c).

Cell Biosynthetic Activity

The biosynthetic capacity of endocervical cells was determined for 12 estrous does before gradient separation. After incubation of cells for 36 h with [3H]NAG, incorporation levels were 0.92 and 0.27 x 10^(-2) cpm/cell, respectively. These values were higher when expressed as counts per minute/presumptive mucous cell (pmc; i.e., vacuolated and granular PAS-positive cells; Fig. 5), a refinement of data warranted by the demonstration that only pmc's incorporated [3H]NAG (see below). Aliquots of heterogeneous cell populations from both estrous and pseudopregnant animals were incubated for 36 h, pelleted onto slides by cytocentrifugation, and stained for histochemical evaluation. A comparison of these preparations with similar preparations of nonincubated cells indicated that the morphological integrity of vacuolated and granular PAS-positive cells was maintained during the incubation period. To verify the association of [3H]NAG incorporation with mucin biosynthesis and secretion, cell sonicates and samples of culture medium were solubilized in Tris-thiocyanate and fractionated by Sepharose 2B column chromatography. The major radiolabeled peak, which was coincident with a peak of protein fluorescence, was excluded from the gel and clearly separated from carrier BSA which nonspecifically bound some of the radiolabel. Epithelial mucins from several sources have been shown to elute in the void volume of Sepharose 2B (48).

In two separate experiments, in each of which two estrous rabbits were used, cells from gradient fractions were incubated with [3H]leucine and [3H]NAG. Isolated cells from throughout the gradient incorporated [3H]leucine (Table I), which verified cell viability after separation, and corroborated conclusions of cell integrity based on trypan blue exclusion. Vacuolated PAS-negative cells did not incorporate significant amounts of [3H]NAG, whereas cell fractions enriched for both vacuolated (fractions 15–17) and granular (fractions 21–28) PAS-positive cells showed mean levels of intracellular [3H]NAG of 1.42 and 2.24 x 10^(-2) cpm/cell, respectively. These incorporation levels were 1.2–2.4 times higher than those obtained for the original heterogeneous populations, which was consistent with the degree of enrichment as determined morphologically. When incorporation data were expressed as cpm/pmc, vacuolated and granular PAS-positive cells showed similar levels of biosynthetic activity (Table II). Furthermore, there was no significant difference in the secretory (extracellular) activity of vacuolated and granular PAS-positive cells when values were expressed as cpm/pmc (Table II).

The biosynthetic and secretory capabilities of endocervical cells from 5-d pseudopregnant animals (two experiments, two animals each) were also evaluated by [3H]leucine and [3H]-NAG incorporation before gradient separation. Isolated cells incorporated [3H]leucine, confirming viability (Table I). The specific activities of endocervical cells in the initial inoculum were 1.34 and 0.43 x 10^(-2) cpm/cell for intra- and extracellular [3H]NAG incorporation, respectively. When expressed as cpm/pmc, the intra- and extracellular specific activity was not significantly higher (P > 0.05) than that of estrous animals (Fig. 5). Cell fractions enriched for both vacuolated (fractions 15–17), and granular (fractions 21–28), PAS-positive cells incorporated [3H]NAG with intracellular levels of 1.32 and 2.08 x 10^(-2) cpm/cell, respectively. The corresponding extracellular levels were 0.67 and 0.93 x 10^(-2) cpm/cell. When results were expressed as cpm/pmc, the biosynthetic and secretory activity of granular cells was not significantly higher than that for vacuolated PAS-positive cells (Table II). Specific biosynthetic and secretory activities were similar for PAS-positive cells from estrous and pseudopregnant rabbits.

The paucity of PAS-positive cells in cell distribution profiles for ovariectomized rabbits was substantiated by the low levels of intracellular (0.24 ± 0.04 x 10^(-2) cpm/cell) and extracellular (0.02 ± 0.01 x 10^(-2) cpm/cell) [3H]NAG incorporation by heterogeneous cell populations from two animals. The low number of mucous cells, displaying a sparse distribution of PAS-positive granules, precluded the calculation of a meaningful value for specific biosynthetic activity.

DISCUSSION

It has long been accepted that cervical secretions undergo hormone-related physicochemical changes which regulate sperm and bacterial access to the upper reproductive tract (6, 27). However, investigators using intact tissue preparations have failed to document unequivocal morphological changes in endocervical epithelia as a function of different hormonal milieu (1, 16, 44). While Hafez (15) demonstrated variability in the type and number of secretory granules in cells from different regions of the cervix, Topkins (44) was unable to correlate similar changes with cyclic alterations in the endometrium. By applying a unit gravity sedimentation procedure to pronase-dispersed endocervical cells, we have now identified, in addition to ciliated cells, three morphologically distinct cell populations (vacuolated PAS-negative, vacuolated, and granular PAS-positive) whose number is modulated by the changing hormonal status of the animal. Using this technique, other investigators have also detected morphologically and functionally distinct subpopulations of cells in rat pituitaries (8) and corpora lutea (47).

The first endocervical cell type (vacuolated PAS-negative) was characterized by the lack of secretory granules and by prominent filopodia and vacuoles which at times contained electron-dense material. This cell type incorporated [3H]leucine but not [3H]NAG. We have considered two possible etiologies for these cells. Firstly, they could be migratory cells of the reticuloendothelial series. Odor (33) has described relatively...
undifferentiated cells in the basal portion of the cervical mucosa and provided ultrastructural evidence that these cells may be migrating lymphocytes or monocytes. In our study, the increase in the percentage of these cells in pseudopregnant rabbits could be attributed to a hormonally (progesterone?) modulated invasion of macrophage-like cells into the uterine stroma and epithelium (34). The possibility that vacuolated PAS-negative cells are reticuloendothelial tissue derivatives seems unlikely, as they do not contain lysosomes and PAS-stainable material (10) or incorporate significant levels of $[^{14}C]$NAG (12). A series of studies aimed at assessing the macrophage-like properties of these cells would include testing...
for specific lysosomal enzymes, for the presence of Fc and C3 membrane receptors by erythrocyte rosetting (11), and for phagocytic activity by challenging the ability of these cells to ingest polystyrene latex beads (5). The second possibility is that vacuolated PAS-negative cells are stem cells. If so, their isolation in enriched populations would provide a unique experimental tool for studying hormone-induced cytodifferentiation and de novo mucogenesis. The question of cell renewal in the endocervical epithelium has been neglected, except for two autoradiographic studies (13, 28) which showed minimal and focal [3H]thymidine incorporation by human endocervical cells. Basal or reserve cells with both mucogenic and squamous potentials have been described in the human cervix where they may represent the progenitors of cervical neoplasia (14, 45). Against a basal cell origin of PAS-negative vacuolated cells lies Odor’s contention (33) that there is no need to postulate the existence of stem cells in the endocervix because differentiated mucous cells are capable themselves of mitotic division.

In this study, two PAS-positive cells, vacuolated and granular, were isolated from estrous, 5-d pseudopregnant and ovariectomized animals. Similar cell types have been described by others in the intact cervices of rabbits, cattle, and monkeys (7, 15). Vacuolated cells have also been observed in the postovulatory and pregnant rabbit (23) and may result from previous
secretory granule-to-granule fusion and mucus release (29). In the absence of estrogen dominance, heterogeneous populations of cells from 5-d pseudopregnant and 16-wk ovariectomized animals showed a significant reduction in the number of PAS-positive cells with a concomitant reduction in the number of mucous granules. Shifts in cell gradient distributions most likely resulted from such a reduction in these granules which altered sedimentation rate by changing cell diameter and/or density (18, 39). In studies using whole tissue, Odor (32) and Riches et al. (41) demonstrated that ovariectomy results in the disappearance of secretory granules from endocervical cells, while the administration of estrogen restores granule populations. It is interesting to note that hormone-mediated quantitative differences in mucous cells have also been reported for a nonreproductive mucous epithelium (17).

To compare the biosynthetic activity of vacuolated and granular PAS-positive cells, incorporation of $[^{14}C]NAG$ into acid-precipitable radioactivity was used as an index of glycoprotein synthesis and secretion. Values represented relative and not absolute rates of incorporation, as the latter required knowledge of precursor pools. In the present studies, mucous cells had linear rates of $[^{14}C]NAG$ incorporation for 48 h (30), and only 1.0% of the available radiolabel was incorporated during the period of isotope exposure. Under these conditions, vacuolated and granular cells were functionally identical. A similar conclusion was reached for morphologically different tracheal cells by Lamb and Reid (21) who used $[^{3}H]$glucose as a mucin precursor. Such results may simply reflect poor resolution, and it is possible that the use of other indicators of mucin synthesis and export or an evaluation of factors (enzymes) involved in the modification of the packaged secretory product (37) may reveal functional differences between the two endocervical mucous cell types. In this respect, it is also worth noting that ultrastructural and histochemical studies indicate microheterogeneity of mucous secretions (19) and thus present the possibility that different cell types may package different secretions.

Ciliated cells represented the fourth isolated endocervical cell type. These cells banded preferentially in the denser regions of the BSA gradient; however, they were also found in fractions enriched for PAS-positive cells. This phenomenon could be caused by size differences and morphological variations (pres-
ence of intracellular canaliculi and random deciliation), and also by the occasional presence of mucous granules. Transdifferentiation between ciliated and mucous cells has been observed in quail oviduct (43), and mucous granules have been described in the ciliated cells of the human cervix (38). In the oviduct of ovariectomized quail, the synergistic effects of estradiol benzoate and progesterone induced populations of both mucous and ciliated cells. However, the increasing of concentrations of estradiol benzoate caused an inhibition of ciliated mucous and ciliated cells. How ever, the increasing of concentrations of estradiol benzoate and progesterone induced populations of both mucous and ciliated cells. These data thus suggest that cells of mucociliary epithelium are capable of morphological modulation in response to the hormonal state of the animal.

Cytodifferentiation in the mammalian endometrium and oviduct requires the sequential availability of estradiol and progesterone (4); genesis of steroid receptors is autoregulated in response to the hormonal state of the animal. Genesis of steroid receptors is autoregulated in response to the hormonal state of the animal. From our study, we would suggest that estrogen provides the stimulus or “triggering influence leading to overt cytodifferentiation” (20) resulting in populations of both vacuolated and granular PAS-positive cells in the cervix of estrous rabbits. When ovarian estrogen availability is altered, i.e., during pseudopregnancy, glycosylation of the secretory product proceeds as usual, as indicated by the unaltered biosynthetic capabilities of the involved cell types. However, it is possible that the reduction in number of mucous cells observed in pseudopregnancy is caused by a decrease in the amount of translational products available for glycosylation.

The data presented here imply the possibility, testable using ovariectomized animals, that steroid hormones regulate cervical mucus secretion by modulating endocervical cell types.

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| Table I | Incorporation of [3H]Leucine into Intracellular (I) and Secreted (S) Proteins |
|---------|-------------------------------|-----------------|-----------------|
|         | Estrous                       | Pseudopregnant   |
| Cell type | I (cpm/cell x 10^-2) | S (cpm/cell x 10^-2) | I (cpm/cell x 10^-2) | S (cpm/cell x 10^-2) |
| Vacuolated PAS-negative | 3.44 ± 0.74 | 1.01 ± 0.57 | 4.23 ± 1.24 | 2.48 ± 1.41 |
| Vacuolated PAS-positive | 5.52 ± 0.59* | 1.62 ± 0.39 | 2.84 ± 0.35* | 2.00 ± 0.51 |
| Granular PAS-positive | 6.13 ± 1.06 | 1.36 ± 0.45 | 6.67 ± 1.70 | 2.00 ± 0.35 |

Values are the mean ± SE.
* Value for estrous animals significantly (P < 0.01) higher than that for pseudopregnant animals, probably because of greater contamination by vacuolated PAS-negative cells. Differences among groups are not statistically significant.

| Table II | Incorporation of [14C]NAG into Intracellular (I) and Secreted (S) Mucin |
|---------|-------------------------------|-----------------|-----------------|
|         | Estrous                       | Pseudopregnant   |
| Animals | Cell fractions | I (cpm/cell x 10^-2) | S (cpm/cell x 10^-2) | I (cpm/cell x 10^-2) | S (cpm/cell x 10^-2) |
| Vacuolated PAS-positive cell | 1.42 ± 0.16 | 0.76 ± 0.16 | 2.49 ± 0.46 | 1.34 ± 0.34 |
| Granular PAS-positive cell | 2.24 ± 0.25 | 1.10 ± 0.26 | 2.43 ± 0.25 | 1.22 ± 0.29 |
| Vacuolated PAS-positive cell | 1.32 ± 0.16 | 0.67 ± 0.10 | 1.91 ± 0.16 | 0.98 ± 0.23 |
| Granular PAS-positive cell | 2.08 ± 0.57 | 0.93 ± 0.21 | 3.56 ± 0.93 | 1.63 ± 0.39 |

* This alternative expression of [14C]NAG incorporation was chosen to correct for the presence of contaminating PAS-negative cells, as these cells do not incorporate significant amounts of radiolabel. Differences among groups are not statistically significant. Values are the mean ± SE.

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