COMPARATIVE CULTURAL CHARACTERISTICS
OF ENDOMETRIAL AND ENDOMETRIOSIS CELLS

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Cultural characteristics of cells from the endometrium and a focus of endometriosis from 35 women before and, in some cases, after treatment with synthetic progestins were compared. Methods of culturing cells of the endometrium and focus of endometriosis were developed. A culture of normal endometrial cells was shown to consist of epithelioid cells, whereas the culture of cells from the focus of endometriosis more frequently had a mixed cell population. Investigation of cultures from a focus of internal endometriosis before and after treatment with infecundin showed no clear difference in cell growth and morphology. Cells from tissue affected by endometriosis showed low mitotic activity and this made the study of the karyotype difficult. The data described are the first relating to culture of cells from a focus of endometriosis.

KEY WORDS: endometrium, endometriosis, cell culture.

The study of the origin and pathogenesis of different forms of endometriosis is an important problem in gynecology [1, 3, 13]. It may be a question of heterotopic differentiation or metaplasia. Heterotopic differentiation can arise as a result of fundamentally different processes: the transformation or transdetermination of cells of initially different types into endometrial cells. The possibility of a genetically determined disturbance of the spatial localization of the stem line of cells giving origin to the endometrial tissue likewise cannot be ruled out.

Fundamental differences between processes leading to the phenomenon of heterotopic differentiation were demonstrated in investigations by Fridenshtein [2]. This worker used a method of cultivation of cells and explants to analyze these processes.

The cell culture technique has the advantage that the cytological features of cell populations are investigated without complication by the regulatory and controlling factors of the organism, and the autonomous potential of the cells constituting these populations can in that way be revealed.

The object of this investigation was to develop methods of cultivating endometrial tissue obtained by curetting the mucosa of the body of the uterus (2-3 days before the expected menstrual period) and tissue from foci of endometriosis in these same patients, and also to study the behavior of the cells in monolayer culture.

EXPERIMENTAL METHOD

Endometrial biopsy and the study of the endometrial tissue were carried out on 35 women, 20 with retrocervical endometriosis, 10 with endometriosis of the ovaries, and five with internal endometriosis. The ages of the women ranged from 25 to 40 years. The endometriosis in 10 patients was treated by a cyclic schedule from the fifth through the 25th day of the menstrual cycle. One tablet each of infecundin and anovlar were given daily.

Endometrium was obtained by curetting the mucous membrane of the body of the uterus in women with various forms of endometriosis. Biopsy material from endometriosis of the ovaries, retrocervical endometriosis, and internal endometriosis was investigated from the same patients. The scrapings of the endometrium and areas of tissue with endometriosis were placed in Eagle's nutrient medium. The cells were cultured in two ways.

Method 1. The pieces of endometrium and endometriosis were washed in Hanks' solution with antibiotics, cut into small pieces with a sterile razor, and the separate pieces were placed on a sterile coverslip in a penicillin flask. A second coverslip was placed above the fragment. The flask was filled with nutrient medium and placed at an angle of 45° (from four to six flasks were set up in parallel). The cultures were incubated at 37°C. Every 2-3 days the culture and the color of the medium were inspected under an inverted microscope. The medium was drawn off after 1-2 weeks and the coverslips with cells growing on them were fixed.

Method 2. The endometrium and tissues affected by endometriosis were washed in Hanks' solution with antibiotics, cut into small pieces with scissors and a razor, and placed in 0.25% trypsin solution. After incubation for 20-30 min at 37°C the trypsin was removed and the tissue was vigorously pipetted into nutrient medium. Immediately after the large fragments had settled the cell suspension was drawn off and transferred to a penicillin flask with a coverslip and to Carrel's flask. After 1 to 2 weeks the coverslips were fixed and the culture in the Carrel's flasks was subcultured in the usual way. The medium was drawn off and a small quantity of heated trypsin added and the flask incubated for a few minutes at 37°C. A cell suspension was formed and transferred to two or three Carrel's flasks, into which fresh nutrient medium was poured. The nutrient medium used had the following composition: Eagle's medium with glutamine 50%, lactalbumin hydrolysate 30%, bovine serum 20%, and penicillin and streptomycin in concentrations of 100 and 50 units/ml, respectively.

The cytological investigation was carried out on intravital preparations (phase-contrast microscopy) and on fixed specimens (Bouin's fixative, staining with hematoxylin-eosin). Whenever possible a cytogenetic investigation was made of the chromosome sets.

Altogether 35 specimens of endometrium and areas of endometrioid heterotopia were cultured. Successful cultures were obtained in 20 cases.

**EXPERIMENTAL RESULTS**

In all cases a zone of growth of epithelial cells appeared after 6-15 days around the explants of endometrium cultured by the first method. The zone of growth was continuous and consisted of polygonal and round cells, in close contact with each other, with granular cytoplasm and a large round nucleus. Many cells with basophilic cytoplasm and with large vacuoles, evidently secretory, were seen in the fixed and stained preparations.

The most characteristic feature of the endometrial cultures obtained by the second method was the colonial growth of the cells. Only in three cases was a continuous layer of epithelioid cells obtained in the first week. Otherwise growth was in the form of colonies. These colonies evidently originated from single cells or aggregates of cells, but not from the separate small fragments of tissue. After incubation for 2-3 weeks the colonies merged to form a cell monolayer. The cells showed the epithelioid character of growth and morphology (Fig. 1). Large cells with large vacuoles in their cytoplasm (a) were frequently found. The cell population of the primary cultures was heterogeneous. Individual groups of closely packed epithelial cells with a small nucleus (c) were observed. Fibroblast-like cells (and fusiform cells in general) were rarely seen as solitary, isolated cells (b).

On subculture the composition of the cell population changed: The number of distinctly epithelioid cells was reduced and the number of fusiform (fibroblast-like) cells increased. More often than not it was impossible to subculture the endometrial cells, for the cells were difficult to remove from the coverslip with trypsin and they failed to grow in the new medium. Cytochemical testing for alkaline phosphatase showed the heterogeneity of the cell population; some cells gave a positive reaction but most cells gave no reaction. The morphology on the X-chromatin body in the endometrial cells was noteworthy: It was definitely smaller in size than in diploid fibroblasts of human skin (Fig. 2).

During culture of cells from a focus of endometriosis by the first method growth of the cells was observed later (after 12-16 days) and it was mixed in character: Typical epithelial growth in the form of "lace" and fusiform fibroblast-like cells could be seen around the same fragment. In two cases (endometriosis of the