HARVESTING AND CULTURE OF EPITHELIAL CELLS FROM HOLLOW ORGANS OF THE FEMALE REPRODUCTIVE SYSTEM

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With 16 Figures in the Text

(Received May 6, 1963)

While primary explants afford a direct method of studying the behavior of cells from biopsies or surgical specimens, they have several disadvantages. The likelihood of contamination of such material is high. In the course of becoming established in vitro, metabolic products from the explanted mass complicate an already complex group of nutritional constituents. While mixtures of epithelial and connective tissue cells may prove interesting, fibroblastic overgrowth may obscure the development of the particular elements under investigation.

Cell lines which can be handled with quantitative techniques generally are highly altered particularly with respect to their karyotype. While cell strains which retain the somatic chromosome number of the species more nearly approximate the prototype, such systems are not easily established from adult human tissue.

It appears, therefore, that disaggregated cells offer special opportunities both from qualitative and quantitative studies, especially when they are harvested from homogeneous tissue complexes. This approach was illustrated by the culture of endothelial cells collected with the use of trypsin from a segment of dorsal aorta of the rabbit (POMERAT and SLICK 1963) and from the vessels of the human umbilical cord (MARUYAMA 1963). Employing this technique, it is possible to harvest epithelial cells from a variety of hollow organs. The purpose of the present study was to describe its application to the female reproductive tract.

Short term experiments on cells from patients may reveal their growth pattern and differentiation in relation to spontaneously or artificially created metabolic conditions. The behavior of normal versus adjacent precancerous and cancerous cells from the vagina in relation to radiation, hormones and various therapeutic procedures also suggest how cell cultures can be employed in gynecological research.

Materials and methods

The materials used in this investigation included the vagina, uterus and oviduct obtained from 6 adult rabbits, 2 adult dogs and from the surgical specimens of 6 patients, ranging in age from 31 to 64 years, who underwent total hysterectomy and salpingectomy for benign uterine diseases.

Initially the method applied by POMERAT and SLICK (1963) to the aorta of young rabbits was employed for all the tissues except the human endometrium and cervical epithelium. Later certain refinements were introduced, as it will be described, for the majority of the experiments.

* This work was performed in the Department of Cellular Biology, Pasadena Foundation for Medical Research, Pasadena, California, and aided in part by a grant from the U. S. Public Health Service, No. 2 G 279 and from the U. S. Army Medical Research and Development Command, Department of the Army, under Research Grant No. DA-MD-49-193-63-G180 administered by C. M. POMERAT.
I. Trypsinization

1. In experimental animals. The uterine cornua, vagina and fallopian tubes were obtained aseptically and severed from one another. After having applied a silk ligature to both ends of the organs, each of them was transferred to a different Petri dish of suitable size, containing Gey’s balanced salt solution in sufficient amount to keep the specimens submerged. The dishes were then brought to the culture room. Such ligatures served to protect the cavity of the viscus from exogenous contamination. Both ligatures were then cut and each organ transferred to another Petri dish containing fresh balanced salt solution. With a sterile syringe and using Gey’s balanced salt solution, free of calcium and magnesium ions (hereafter referred to as BSS), the debris, mucus and blood were washed out of the viscus. The ends of the organ were then fitted over the blunt metal tips of two sterile 10 ml syringes, one of which was empty and the other containing 5 ml of a 0.5% solution of trypsin in BSS. A silk ligature was applied to both ends of the organ and around the tips of the syringes as tightly as possible, so as to prevent leakage of the enzyme solution. The free ends of each silk tie were taped to the barrels of the syringes to prevent the ties from sliding off (Fig. 1).

By gently depressing the plunger of the syringe a small amount of the trypsin solution was transferred into the lumen of the organ until its wall was seen to be slightly distended. The trypsin solution was then removed from the organ into the syringe. This manipulation was repeated several times at intervals of about 5 min. This process was conducted at room temperature for a period of 30 to 40 min. The time required depended upon the degree of turbidity observed in the fluid in the syringe. More recently this technique was slightly modified. One of the silk ties originally applied to one end of the hollow organ was left in place. The other ligature was removed in the culture room and, after having washed the lumen of the viscus with BSS, the patent end of the organ was fitted over a sterile two-way metal stopcock and secured to it with a silk tie. A sterile 10 ml syringe containing 5 ml of the trypsin solution was then fitted over the inlet of the stopcock and a 15 gauge needle was applied to the free outlet (Fig. 2). Obviously the small caliber of the rabbit and dog oviducts required the use of a small blunt needle.

By depressing and withdrawing the plunger of the syringe, at time intervals of about 5 min, the enzyme solution could be transferred periodically to the lumen of the organ to act upon the epithelial lining. Samples of the solution were easily obtained by switching the stopcock toward the needle's outlet and collected in a test tube or deposited on a slide for microscopical examination. Moreover, it was possible to introduce a fresh warm solution of the enzyme periodically and to carry on the procedure for a long time without risk of damage to the cells. Fresh fluid was presumed to be more active in dissociating basal cells. Samples of cells in the trypsin solution were collected through the needle into conical centrifuge tubes for culture. This modified technique proved to be very satisfactory and in fact was almost exclusively used later with all the organs that were investigated.

2. Human material. Surgical specimens were quickly transferred from the operating suite to the culture room. Two silk ties were applied to the uterine