ORGAN CULTURE OF THE RAT'S UTERUS

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The organ culture method can now be used for the cultivation of many different organs and tissues from adult animals and man. Good survival in organ culture conditions can be obtained with the endocrine organs and hormone-dependent tissues: pituitary, ovary, thyroid, parathyroid, mammary gland, seminal vesicles, prostate; moreover, if the appropriate hormones are added to the nutrient medium, the cultivation of these organs is imporved and they may even function.

Because the growth of the organs listed above in organ cultures is dependent on hormones, this means that the organ culture method can be used to study the hormone regulation of proliferative and secretory processes in the tissues and organs, which is important for understanding the mechanism of the antitumor action of hormones. Such an investigation in organ culture conditions has advantages over investigation in the intact organism, for the effect of compensatory reactions of the endocrine system on hormonal effects can be excluded.

Little information about organ culture of the uterus can be found in the literature. The first attempt to cultivate the uterus of newborn and adult rats was made by Trowell [6]. Later, Everett [4] cultivated the embryonic guinea pig uterus and studied the mitotic activity of the epithelium.

The object of the present investigation was to study the effect of hormones on the proliferative and proliferative and secretory processes in the endometrium of rats of various ages in the conditions of organ culture of the uterus.

EXPERIMENTAL METHOD

The uterus of noninbred rats aged 5, 10, 20, and 30 days was cultivated for 3 and 7 days by Chen's method [2] as modified by R. Kh. Adil'gireeva [1] for adult tissue. Cultivation was carried out in watch glasses on a cigarette paper raft, in a nutrient medium consisting of synthetic medium No. 199 (50%), calf serum (25%), and chick embryonic extract in a dilution of 1:2 in physiological saline (25%). In addition glucose was added to the nutrient medium: 1-2 drops of a 40% solution was added to 10 ml of medium No. 199. The hormone preparations were dissolved in acetone and then suspended in medium No. 199 so that the acetone concentration in the medium did not exceed 0.5-1.0 %. Dihydrostilbestrol was used in a dose of 0.5 and 5.0 μg/ml nutrient medium. The watch glasses were placed in Petri dishes in a moist atmosphere, the gaseous phase of the culture was blown out with carbon dioxide, and the specimens were then incubated in a moist chamber at 35±0.5°.

The uterus was taken from animals killed by decapitation. The uterine cornua were freed from connective tissue and cut into disks measuring 1.0-1.5 mm. Five or six disks were placed on each raft. After 3-4 days the cultures on the raft were washed in medium No. 199, and then transferred on the same raft into fresh nutrient medium. After the end of the experiment the culture was fixed in Bouin's fluid, taken from the raft, and treated by the usual histological methods. The sections were stained with hematoxylin-eosin.

EXPERIMENTAL RESULTS

The study of the histological preparations showed that the uterus of the 5- and 10-day old rats survives readily in organ culture conditions for 3 and 7 days and preserves its typically uterine structure. In the structure of the layers of its wall the uterine explant (see figure, b) differed very little from the

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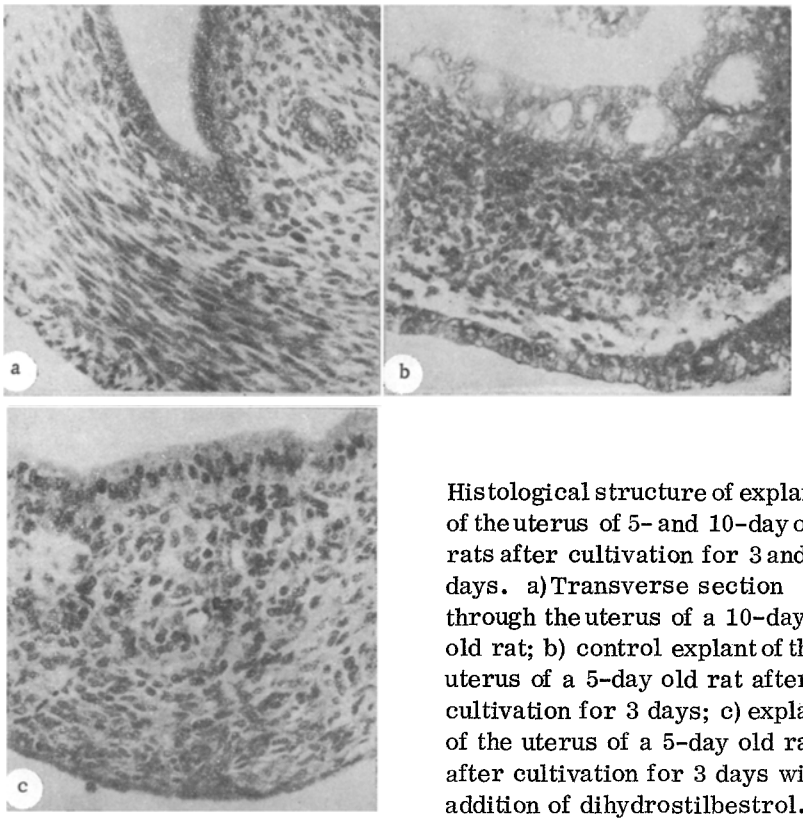

Histological structure of explants of the uterus of 5- and 10-day old rats after cultivation for 3 and 7 days. a) Transverse section through the uterus of a 10-day old rat; b) control explant of the uterus of a 5-day old rat after cultivation for 3 days; c) explant of the uterus of a 5-day old rat after cultivation for 3 days with addition of dihydrostilbestrol. Hematoxylin–eosin. 270 ×.

uterus of rats of the same age (see figure, a). In the uterine explants from 5- and 10-day old rats after cultivation for 3 and 7 days, for example, two layers of the uterine wall – the endometrium and myometrium – were clearly distinguishable. In contrast to the uterine epithelium of the rats of this age (see figure, a), the endometrial epithelium of the uterine explant was higher, and in some places its cytoplasm contained numerous vacuoles or resembled the apocrine type. Mitoses were clearly visible in the epithelium. As a rule the epithelium grew out of the uterine canal and covered the cultivated fragment on its surface (see figure, b); a new membrane of endometrial epithelium was formed around the explant.

A wide connective-tissue membrane of the endometrium, rich in cells, could be seen under the epithelium of the central canal of the uterine cornu. The myometrium in the uterine explants was also well developed, although the regular arrangement of the muscle cells was disturbed in the course of cultivation, as may be seen especially clearly in the cultures of the uterus of the 10-day old rats (see figure, b),, for in animals of this age differentiation into longitudinal and circular muscle layers is distinctly visible. The third layer of the uterine wall – the serous membrane – was ill defined in the organ cultures and sometimes could not be seen because of the formation of the new membrane around the explant from the endometrial epithelium growing out of the central canal of the uterine cornu.

The uterus of 20- and 30-day old rats tolerated the conditions of organ cultivation for 3 and 7 days much less radily, for the typically uterine structure was preserved only in individual explants, and most commonly only in part of the explant. In most explants of the uterus of rats of this age the epithelium in the central canal of the cornu died, and only the epithelium on the surface of the explant survived. The connective-tissue cells of the endometrium also died. The layer of myometrium, on the other hand, hypertrophied considerably and formed the major part of the explant.

During cultivation of the uterus of 5- and 10-day old rats with the addition of hormones in the doses mentioned above to the nutrient medium, no reaction of the uterus could be detected to dihydrostilbestrol alone, to progesterone alone, or to a combination of both (see figure, c). No stimulation of mitoses or of secretory changes in the epithelium of the endometrium, for example, could be observed after the addition of hormones to the nutrient medium. The vacuolation of the cytoplasm of the epithelium and its conversion to aprocrine type, as described above, were equal in degree in the control and the experimental cultures.

Areas of stratified epithelium, fan-shaped papillae of epithelium with goblet cells—changes developing in the endometrium of the uterus in animals receiving estrogens—also were observed in both the experimental and the control cultures.

These changes in the morphology of the control uterine explants thus show that explantation of the uterus itself produces the changes in the endometrium arising in vivo after administration of estrogens or other procedures [5]. The possibility is not ruled out that the reaction of the endometrium to explantation prevented to some extent its reaction of hormones. However, the author suggests that in these experiments the absence of changes in the endometrial epithelium to administration of hormones was not the result of creating conditions of cultivation in which the uterine cornu could survive readily in the absence of hormones also. Support for this suggestion is given by data on the organ cultivation of mammary gland tissue [3], according to which the reaction of this tissue to hormones was observed during cultivation on a minimal nutrient medium.

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