Hormonal control of pinocytosis in the uterine epithelium of the rat

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**Summary.** Ovariectomized rats were treated with oestradiol-17β and/or progesterone to mimic the hormonal parameters inducing uterine sensitivity for implantation. The degree of pinocytosis of trypan blue and ferritin in the endometrial cells was examined. Significant epithelial pinocytosis of trypan blue occurred after a 3-day treatment of progesterone, and uptake was independently increased by priming with oestrogen and by oestradiol given on the 3rd day of progesterone treatment. Progesterone treatment caused uptake of ferritin by the epithelial cells; in control animals epithelial and stromal cells were involved. Oestrogen priming enhanced ferritin absorption, while 'nidatory' oestrogen had no effect. Oestradiol given alone completely blocked pinocytosis of both intraluminally injected substances.

**Introduction**

The endocytic properties of the rat uterine epithelium were first indicated by the demonstration that this tissue is capable of absorbing colloidal dye particles from the lumen and of concentrating them into the cytoplasm as large granules (Vokaer, 1952). Since this phenomenon could only be observed during the progestational period, it was thought to be under hormonal control and related to implantation (Vokaer, 1952; Vokaer & Leroy, 1962).

Various aspects of the temporal and quantitative relationships between ovarian secretions and endometrial receptivity have been studied in rats and mice (see Finn & Martin, 1974; Psychoyos, 1974). The hormonal pattern involved can be divided into three main components. To produce maximal decidualization, the uterus must first be subjected to a relatively high oestrogen concentration; after 3 days a considerable amount of progesterone is required for at least 48 hr before small quantities of so-called 'nidatory' oestrogen induce complete uterine sensitivity and implantation can occur. This hormonal sequence can be easily reproduced by giving exogenous sex steroids to ovariectomized animals.

We have attempted (i) to define the contribution provided by each of the three hormonal phases for the production of uterine sensitivity to the degree of epithelial pinocytosis, and (ii) to determine whether the endocytic behaviour of uterine epithelial cells would vary towards different chemical substances.

**Materials and Methods**

Wistar rats (±150 g body weight) were anaesthetized with bromoethanol (Bayer Products) and ovariectomized. Seven days later groups of 6 animals were allocated to the various hormonal treatments outlined in Table 1. A laparotomy was performed 48 hr before autopsy and 50 µl 1% trypan blue (Geigy) in saline were injected into one uterine horn while the other horn was similarly treated with 5% aqueous ferritin (Taab Laboratories). A transverse ligature was placed above the cervix to prevent reflux. The horns were removed by amputating them at least 5 mm above the ligature to avoid interference of uterine trauma with the pinocytosis (Vokaer & Leroy, 1962). The trypan
blue-injected horns were fixed in Bouin's fluid and those instilled with ferritin were placed in 40% formalin. After embedding in paraffin wax transverse sections were cut at 6 µm and Perl's histochemical method for ferric iron (Pearse, 1960) was applied to the ferritin-treated material. All sections were counterstained with Nuclear Fast Red.

Table 1. The effect of different hormone regimens on the pinocytotic uptake of trypan blue and ferritin by the uterine epithelium in ovariectomized rats

<table>
<thead>
<tr>
<th>Groups</th>
<th>Days of treatment</th>
<th>Degree of pinocytosis*</th>
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<tbody>
<tr>
<td></td>
<td>1 2 3 4 5 6 7 8 9 10</td>
<td>Trypan blue</td>
</tr>
<tr>
<td>I</td>
<td>— — — — — — — —</td>
<td>K</td>
</tr>
<tr>
<td>II</td>
<td>E E — — — — —</td>
<td>—</td>
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<tr>
<td>III</td>
<td>— — — — — — P P</td>
<td>P K</td>
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<tr>
<td>IV</td>
<td>— — — — — P P + e</td>
<td>P K</td>
</tr>
<tr>
<td>V</td>
<td>E E — — — P P</td>
<td>P K</td>
</tr>
<tr>
<td>VI</td>
<td>E E — — — P P + e</td>
<td>P K</td>
</tr>
</tbody>
</table>

Day 1 is the 7th day after ovariectomy; E = 500 ng oestradiol-17β; P = 3 mg progesterone; e = 50 ng oestradiol-17β (all hormones were injected s.c. in arachis oil); 50 µl 1% trypan blue and 5% ferritin were injected into left and right uterine horns respectively, 48 hr before killing (K).

* The results are expressed as a total score for all 6 rats in a group: maximum score = 12. See text for scoring methods.

Although the degree of pinocytosis could not be measured exactly, we attempted to estimate it as objectively as possible. For each uterine horn, two or three levels of section were examined under a microscope at a magnification of ×500. Since the appearance of pinocytotic epithelia was similar at all the levels, a score could be given for each animal as follows: 0—absence of detectable pinocytosis or only a few tiny grains in some epithelial cells (Pl. 1, Fig. 1); 1—conspicuous vesicles of pinocytosis located at the apical region only of almost all cells (Pl. 1, Figs 3 and 4); 2—abundance of large pinocytotic inclusions throughout the cytoplasm of all cells (Pl. 1, Figs 5 and 6).

In instances when the choice between two adjacent scores was difficult, the highest possible mark was systematically attributed. The sections were independently scored at random by two observers ignoring the treatments involved. As the two scores were always consistent, the method was assumed to give a reliable assessment of the degree of pinocytosis. Experimental groups were reasonably homogeneous since, among identically treated animals, the difference between extreme individual scores never exceeded one point, i.e. scores of 0 and 2 were never recorded in the same group.

Results and Discussion

The results in Table 1 confirm previous observations (Sartor, 1969), indicating that progesterone is necessary to obtain conspicuous pinocytosis of trypan blue particles into the rat uterine epithelium. In a pilot experiment we also found that at least 3 days of treatment with high doses of progesterone

EXPLANATION OF PLATE
Sections of the uterus of ovariectomized rats.

Fig. 1. Untreated control (Group I): pinocytosis of trypan blue is hardly visible.

Fig. 2. Untreated control (Group I): there is significant epithelial pinocytosis of ferritin and stromal extension of pinocytosis (arrows).

Figs 3 and 4. Animal treated with progesterone alone (Group III): there is conspicuous pinocytosis at the apical region of epithelial cells in the trypan blue- (Fig. 3) and ferritin- (Fig. 4) injected horns.

Figs 5 and 6. Animal injected with oestradiol and progesterone (Group VI): heavy epithelial pinocytosis extends throughout the cytoplasm in the horn in which trypan blue (Fig. 5) and ferritin (Fig. 6) were injected.
are required to elicit visible epithelial endocytic activity. With a shorter duration of treatment or a lower dose (<1 mg/day), only a few faint grains of dye were seen in the apical cytoplasm of some cells, an appearance similar to that observed in ovariectomized controls (Group I; Pl. 1, Fig. 1).

From the results for Groups IV and V, pinocytosis of trypan blue in the progesterone-dominated uterus is independently stimulated by the injection of 50 ng oestradiol-17β on Day 8 and by priming with oestrogen on Days 1 and 2. Pinocytotic activity was augmented in animals treated with both oestrogen regimens (Group VI). These findings can be correlated with ultrastructural observations indicating that both oestrogenic determinants of uterine sensitivity modify the properties of the apical membrane of the epithelial cell (Finn, 1974). Under the influence of progesterone alone, the microvilli of apposed epithelial cells interdigitate in a simple manner, whereas a small dose of oestradiol results in a complex configuration referred to as the second stage of uterine closure (Pollard & Finn, 1972). Priming with high doses of oestradiol delays these modifications of the uterine luminal surface which normally occur at the end of the gestational phase (Finn & Pollard, 1973). The causal connection between such an inhibitory effect and the subsequent enhancement of endocytic capacity in epithelial cells treated with progesterone is an interesting aspect of the action of sex steroids on membrane function.

In our material, there was no significant extension of pinocytosis to the underlying endometrial stroma in the trypan blue-treated uteri. This finding apparently contradicts the observations of Sartor (1969), but the experimental conditions of the two studies were different; Sartor observed stromal pinocytosis in animals treated daily with 5 mg progesterone plus 0.1–1.0 μg oestradiol-17β. In previous investigations (Vokaer, 1952; Vokaer & Leroy, 1962), it was found that intracellular accumulation of trypan blue remained in the epithelium, except in the vicinity of localized uterine trauma. It remains to be determined whether the change of basal membrane permeability, allowing the passage of pinocytosed material towards the stroma, bears any relationship to the capacity of transfer through the epithelium of the information which normally initiates the decidual reaction (Finn, 1974).

The results from the injection of uteri with ferritin differed from those obtained with trypan blue since ferritin was absorbed by the uterine epithelium in control and hormone-treated animals. Conspicuous pinocytosis was present in the stroma of control rats (Pl. 1, Fig. 2), but it could not be determined if ferric material had been transferred through the epithelium directly or had been picked up from the lumen by macrophages which subsequently migrated. In progesterone-treated animals, ferric iron was detected only in the epithelial cells, a picture similar to that seen after injection of trypan blue. Priming with oestrogen (Group V) apparently favoured ferritin pinocytosis, whereas the administration of 50 ng oestradiol-17β had no detectable effect (Group IV).

High doses of oestradiol given alone (Group II) completely blocked the endometrial capacity for pinocytosis since neither injected substance could be visualized at all, either in the epithelium or in stromal cells. This observation is consistent with electron microscope studies showing that the oestrogen-stimulated uterine epithelium is involved mainly with growth and secretory activity (Nilsson, 1959), leading to accumulation of fluid into the lumen (Homburger & Tregier, 1957). Under the influence of progesterone and during the gestational period, the membranes and cytoplasmic organelles of uterine epithelial cells undergo several changes suggesting strong resorptive activity (Löof, Nilsson & Toss, 1968; Ljungkvist, 1971), which is in keeping with our data on pinocytosis (Table 1, Groups III to VI).

In a recent review, Finn (1974) has discussed arguments leading to the conclusion that initiation of implantation results from subtle molecular changes at the luminal membrane of uterine epithelial cells. As shown in the present work, the occurrence in these cells of pinocytosis which is obviously characteristic of apical membrane function, is related to the same hormonal parameters as those required for implantation. This property of the uterine epithelium may therefore prove a useful model for gaining further insight into the mechanism of implantation.

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References


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