Autoradiographic Study of the *in Vitro* Uptake of Estradiol by Eosinophils in Human Endometrium[1] (35523)

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(Introduced by W. E. Stumpf)

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Uterine eosinophils of the rat take up 6, 7-^{3}H-estradiol-17β in an *in vitro* system (1–3). Neither progesterone-7α-^{3}H nor testosterone-7α-^{3}H are taken up by these cells (3). The *in vitro* uptake capacity of each uterine eosinophil has been found to vary inversely with the blood level of estrogens in the rat, and is attributed to competition for the same acceptor site between 6,7-^{3}H-estradiol-17β of the incubation medium and the tissue level of endogenous estrogens (3). The eosinophilic acceptor site has the following characteristics: (a) great affinity for estradiol-17β, (b) high specificity for estrogens but not for other steroid hormones, and (c) a limited capacity for the uptake of estradiol due to a finite number of binding sites (3). The number of uterine eosinophils in the rat varies according to the hormonal state of the animal, and is directly related to the level of estrogens in the blood and decreased by progesterone (1, 3–8). These observations suggest that uterine eosinophils have a physiological role in the mechanism of action of estrogens (3).

The present communication reports the *in vitro* uptake of tritiated estradiol by eosinophils of the human endometrium.

Methods. Endometrium biopsies from 40 women (between 16 and 40 years of age) without gynecologic or endocrine pathology were obtained by curettage. The samples were removed in different phases of the menstrual cycle and processed by one of the following two procedures: (i) Samples were frozen in O. C. T. (Lab-Tek, Westmont, Illinois) and sectioned in a cryostat. Six to 8-μ sections were incubated for 10 min at 20° in a phosphate buffer solution containing 0.1 to 1.0 μCi/ml of 6,7-^{3}H-estradiol-17β (sp act 38.1 Ci/mmole), and autoradiograms were prepared as previously described (3). (ii) Immediately after biopsy, tissue samples were incubated for 30 min in a buffered solution containing 0.5 to 5.0 μCi/ml of tritiated estradiol and 0.01 to 0.1% of H_2O_2. The addition of hydrogen peroxide to the incubation medium greatly increases the *in vitro* binding of tritiated estradiol which is firmly bound, so that the tissue can be treated with organic solvents prior to embedding with paraffin (2). Following incubation, the samples were washed in 10 changes of isotonic saline, embedded with paraffin, and processed for autoradiography. The two techniques used did not reveal any difference in the uptake of estradiol by the eosinophils.

The chemical nature of the radioactivity in the eosinophils after the incubation with labeled estradiol has not been identified. Indirect evidence indicates this to be estradiol (1–3).

Results. Human endometrial eosinophils bind 6,7-^{3}H-estradiol-17β in an *in vitro* system previously described for the rat (1–3). Figures 1 and 2 show representative autoradiograms of uptake of tritiated estradiol by human uterine eosinophils. Their cytoplasm

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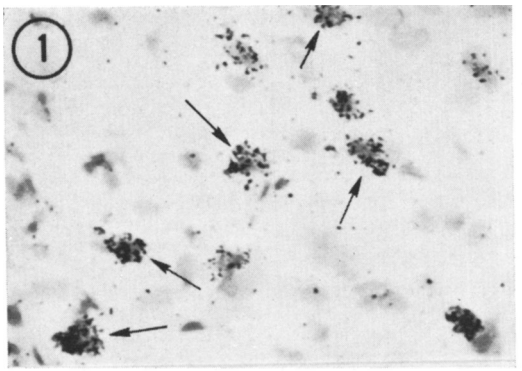

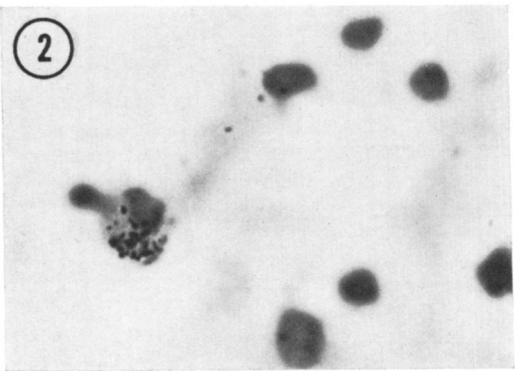

FIGS. 1 and 2. Autoradiograms of human endometrium after incubation with 6,7-^{3}H-estradiol-17β showing incorporation by uterine eosinophils (arrows): exposure time, 7 days; stained with hematoxylin and eosin, 6 μ; (1) ×480; (2) ×1200.

incorporates the hormone in a considerably greater proportion than does the rest of the uterine tissue. It was observed that the endometrial eosinophils are abundant in the second week of the cycle, less abundant in the first and third weeks, and relatively scarce in the last week of the cycle. A qualitative evaluation of autoradiograms of endometrial biopsies removed on different days of the menstrual cycle suggests that the uptake of estradiol by uterine eosinophils is greater at those times during the cycle when endogenous estrogen production is relatively lower, *i.e.*, during the first and last weeks of the cycle.

Discussion. The present work shows that human endometrial eosinophils bind 6,7-^{3}H-estradiol-17β under conditions similar to those previously described for the rat (1–3). Although a quantitative study was not made, a qualitative evaluation of endometrial biopsies suggests that the uptake of estradiol by each eosinophil is greater at the first and last weeks of the cycle. This would correspond to the data obtained from the rat (3); however, a quantitative study is required for the human before a definitive statement is made.

Uterine eosinophils might mediate some effects of the estradiol on the uterus (3). Although a two-step mechanism for the interaction between estradiol and the rat uterus has been proposed (9, 10), one can also speculate that the 8S–5S binding system (9, 10) and the eosinophil binding system (1–3) may represent different mechanisms of estrogen action. The 8S–5S binding mechanism has been studied extensively by means of biochemical techniques (9, 10), and predominantly nuclear localization in epithelium, substantia propria, and muscularis has been observed by autoradiographic techniques (11, 12). These studies were carried out, however, with immature or ovariectomized rats, which do not have eosinophils. In contrast, in the uterus of mature rats in different hormonal conditions, the eosinophil binding system has been demonstrated *in vitro* by means of autoradiography (1–3). The absence of nuclear concentration of estradiol in uterine tissue in these experiments may be attributable to the extraction of estradiol from the nuclear binding sites during the experimental procedure. In this context, it may be of interest that a 4S binding protein has been reported in uterus of mature rats, which is lost after ovariectomy (13). The hypothesis of two different estrogen-binding systems, based on differences in the intensity of effects of various estrogenic substances, has also been proposed by Hechter and Halkerston (14) who consider at least two kinds of "reactive sites" in the uterus, which would represent different cell types.

The contraceptive action of intrauterine devices (IUD) may be explained on the basis of present results. One can postulate that the IUD causes local mechanical irritation with consequent release of histamine and increase in the local number of eosinophils. The latter may be responsible for a greater local estrogenic effect which would explain the lack

of ovum implantation. This is based on preliminary evidence from our recent studies, which show that the IUD in rats causes a local increase in the number of eosinophils in the uterus, principally located in the chorion adjacent to the epithelium of the endometrium.

Summary. Human uterine eosinophils from endometrial biopsies have been demonstrated to take up tritiated estradiol, in an *in vitro* system previously described for the rat. The eosinophil-estrogen binding system is considered to be involved in the mechanism of estrogen action in the uterus, in addition to the 8S–5S binding system.

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