Effects of Intrauterine Foreign Body on the Localization of [^{3}H-]Estradiol in Rat Uterine Tissues, Polymorphonuclear Neutrophiles and Eosinophiles[1] (37156)

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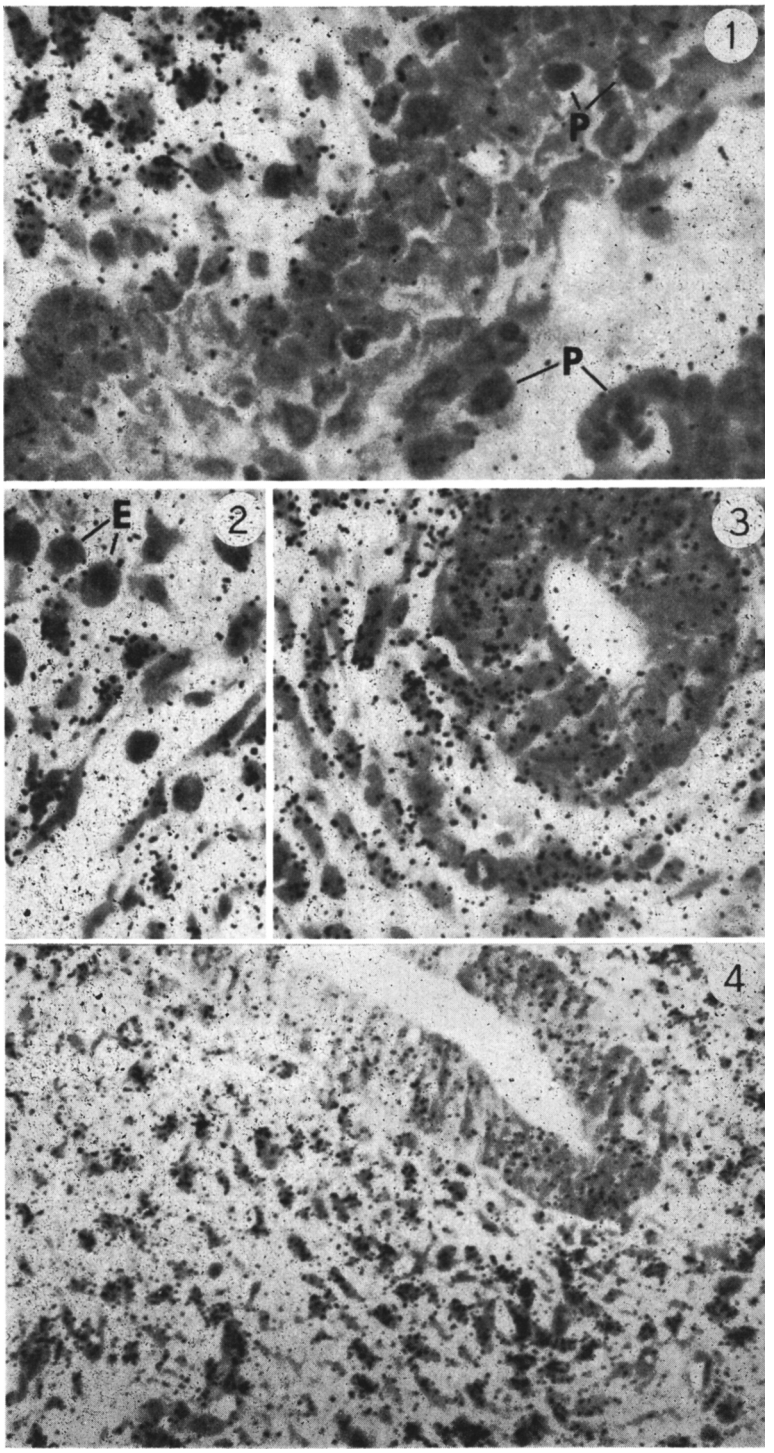
In rats, the presence of an intrauterine foreign body (IUFB) is known to suppress uterine responses to ovarian hormones (1–4). Although the manner in which an IUFB interferes with the action of ovarian hormones has not been completely elucidated, biochemical studies in rats suggest that it stimulates the uterine uptake of [^{3}H]-estradiol (5), but it does not alter the uterine uptake and disappearance of radioactivity of injected [^{3}H]-progesterone (6). However, these studies do not indicate whether IUFBs alter the regional or subcellular distribution of ovarian hormones in the uterus. It has been demonstrated that the eosinophils in the uterus accumulate significant amounts of [^{3}H]-estradiol under certain *in vitro* conditions (7, 8). Since IUFBs are known to promote inflammation in the rat uterus (9), it is possible that the infiltrating leukocytes or other inflammatory cells may concentrate and/or metabolize steroid hormones and consequently reduce their availability for the uterine target cells. The following studies were, therefore, undertaken to determine the effects of an IUFB on cellular and subcellular localization of [^{3}H]-estradiol in uterine cell types.

Materials and Methods. Two and one-half month old female rats of the Holtzman strain were bilaterally ovariectomized and a 5-0 silk suture was placed throughout the length of the lumen of the right uterine horn (IUFB horn), leaving the contralateral left horn (control horn) intact. Two weeks later seven animals were injected subcutaneously with 1 μg per day of estradiol-17β dissolved in corn oil, for a period of 4 days. Forty-eight hours after the last steroid injection, six animals were injected subcutaneously with 0.1 μg/100 g body weight of 2,4,6,7-[^{3}H]-estradiol-17β (specific activity 95 Ci/m*M*, New England Nuclear, Boston, Mass.), dissolved in physiological saline. These animals were killed by decapitation 10 and 60 min after injection of the radioactive estrogen. In addition, one animal was killed 2 min after intravenous injection in order to study the possible uptake of [^{3}H]-estradiol in eosinophils at an earlier time interval. At autopsy, the control and IUFB uterine horns were excised. Tissues were placed on tissue holders and frozen in liquified propane at —180°. Sections, 2 μ thick, were cut in a cryostat (Harris Manufacturing Company, Cambridge, Mass.), and freeze-dried in a cryosorption pump (Thermovac Industries Corp., Copiague, New York) within the cryostat. The freeze-dried, unfixed and unembedded sections were dry-mounted on photographic emulsion (Kodak NTB 3) precoated and desiccated slides. After three to six months of exposure at —15°, the autoradiograms were developed and stained either with hematoxylin and eosin, or with methylgreen pyronin. Details of the dry-autoradiographic technique have been described (10).

Results. The autoradiograms of the IUFB horn, prepared 10 and 60 min after [^{3}H]-estradiol injection, did not show nuclear concentration of radioactivity in the cells of the multilayered metaplastic lumenal epithelium, especially the outer layers (Fig. 1). The cells

[1] Supported by a grant from the Rockefeller Foundation to the Laboratories for Reproductive Biology, University of North Carolina, Chapel Hill, North Carolina, and by Grant Number M68-155 of the Population Council, New York, to the Southwest Foundation for Research and Education, San Antonio, Texas.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of the basal epithelial layer showed lesser concentration of radioactivity than the glandular epithelium and labeled stroma cells (Figs. 2 and 3). Also a lesser concentration of radioactivity existed in the substantia propria cells underlying the lumenal epithelium (Fig. 1), while high nuclear concentrations could be seen in other stromal cells, gland cells and muscularis. In the control horn, nuclear concentration of radioactivity was observed in cells of the lumenal and glandular epithelium, substantia propria (Fig. 4) and muscularis. Polymorphonuclear leukocytes, which were found to be accumulated in the IUFB horn but not in the control horn, did not concentrate radioactivity. In the IUFB horn, leukocytes were abundant in the uterine lumen, the metaplastic lumenal epithelium and the substantia propria. Eosinophiles were found mainly in the deeper layers of the substantia propria. While eosinophiles were seen also in the control horn, they were more frequent in the IUFB horn. None of the eosinophiles showed concentrations of radioactivity at 2, 10, and 60 min after [^{3}H]estradiol injection (Fig. 3).

Discussion. The present autoradiographic studies demonstrate a nuclear concentration of radioactivity in cells of the glandular epithelium, substantia propria and muscularis of the uterus with or without IUFB after the injection of radioactively labeled estradiol. While the lumenal epithelium of the control horn showed a nuclear concentration of radioactivity, the cells of the hyperplastic multilayered lumenal epithelium of the IUFB horn were essentially free of radioactivity, except for a few silver grains overlying the basal layers. The typical nuclear concentration of radioactivity observed in uterine cells in the present experiment is in agreement with the findings that were reported for immature intact and mature ovariectomized rats (11) after the injection of [^{3}H]estradiol. It is not understood, however, why an IUFB should influence the retention or concentration of estrogen in the lumenal epithelium but not in other cells of the uterus. Studies which have been carried out by others, demonstrate that IUFBs promote hypertrophy and hyperplasia in lumenal epithelium (12, 13). Also, it has been shown that the exposure of rat uterus to a foreign body during Days 3–4 post coitum markedly stimulates the mitotic activity in lumenal epithelium (4). It is possible that the ability of lumenal epithelial cells to concentrate ovarian hormones is reduced or lost as a result of alterations in the pattern of cellular growth and division. In this respect, the situation in the IUFB horn may be analogous to that in the rat vagina, where estrogen uptake and binding is markedly low or absent in the outer cornifying layers of the epithelium (14). The lack of accumulation of radioactivity in infiltrating polymorphonuclear leukocytes and eosinophiles after the injection of labeled estradiol suggests that these white blood cells are not "target" cells for estradiol, but are attracted and influenced indirectly by actions of the hormone.

The present *in vivo* observations do not support the results obtained by *in vitro* studies (7, 8) in which radioactivity was found to be concentrated exclusively in eosinophiles but not in other uterine cells.

Summary. Effects of unilateral placement of an intrauterine foreign body (IUFB) on the localization of [^{3}H]estradiol were studied in ovariectomized estrogen-primed rats, using dry-autoradiography. Autoradiograms of control and IUFB horns were prepared at 2, 10 and 60 min after [^{3}H]estradiol administra-

FIG. 1–4. Dry-autoradiograms of uterine tissues 1 hr after subcutaneous injection of [^{3}H] estradiol-17β. In the IUFB-horn (Figs. 1–3), the nuclei of the metaplastic lumenal epithelium and subepithelial cells (Fig. 1) retain no or very little radioactivity when compared with the glandular epithelium (Fig. 3) and substantia propria cells (Figs. 1–3). Polymorphonuclear leukocytes (P) and eosinophiles (E) do not concentrate and retain radioactivity. In the control horn (Fig. 4), contrary to the IUFB-horn, the lumenal epithelium concentrates radioactivity. All sections are 2 μm thick, exposed for 157 days, stained with methlygreen pyronin (Figs. 1, 3, and 4) or H and E (Fig. 2). ×800 (Figs. 1–3), ×430 (Fig. 4).

tion. In the control uterine horn, nuclear concentration of radioactivity was observed in cells of the lumenal and glandular epithelium, substantia propria and muscularis. In the IUFB uterine horn, no nuclear concentration of radioactivity was observed in the cells of the outer layers of the multilayered metaplastic lumenal epithelium, but little radioactivity existed in cells of the basal layer of the lumenal epithelium and the underlying cells of the substantia propria with higher nuclear concentrations in the stromal and glandular cells. Infiltrating polymorphonuclear leukocytes and eosinophiles did not accumulate radioactivity. These observations suggest that an IUFB alters uptake of estrogen in proliferating epithelial cells adjacent to the uterine lumen, and that the infiltrating white blood cells are not target cells for estradiol.

We thank Gerda Michalsky and Anu Turnbull for technical assistance.

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Received Nov. 8, 1972. P.S.E.B.M., 1973, Vol. 142.