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AUTORADIOGRAPHY OF MAMMARY GLANDS AND UTERI OF MICE AND RATS AFTER THE INJECTION OF [^{3}H]-ESTRADIOL*

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SUMMARY

The distribution of radioactivity in mammary glands and uteri of lactating mice and rats, and in mammary glands of pregnant rats was studied 1 hr after the injection of [2,4,6,7-^{3}H]-estradiol-17β, using drymount autoradiography. In mammary glands of lactating animals radioactivity was found to concentrate in nuclei of epithelial cells of acini and ducts, while myoepithelial cells and fat cells did not retain radioactivity. In the same animals the uterine tissues also showed nuclear concentration of radioactivity in stromal, glandular and muscle cells, with little or no concentration of radioactivity in luminal epithelial cells. In pregnant rats on day 7 and 13 a weak concentration of radioactivity was observed in epithelial cells of the acini and ducts of the mammary gland. The autoradiographic results demonstrate that certain tissues of the mammary gland are targets for estradiol, similar to the uterus.

Gonadal steroids promote mammary growth indirectly by stimulating the secretion of prolactin and growth hormone in the anterior pituitary [1] and directly by acting on mammary tissue. Autoradiographic studies with tritiated thymidine showed that estradiol promotes growth of interlobular ducts and end buds [2].

Little information is available about the cellular and subcellular concentration of estrogen in the mammary gland. After the injection of a "physiological" dose of 0.1 μg per 100 g body weight of [6,7-^{3}H]-estradiol-17β into ovariectomized C3H mice, the mammary gland has been shown to concentrate and retain estradiol against a large concentration gradient to the blood [3]. In the rat, mammary gland accumulated more tritiated estradiol than did skeletal muscle and fat [4]. DMBA-induced estrogen-dependent mammary tumors in rats also retained [^{3}H]-estradiol-17β as shown by radioassay [5, 6] and autoradiography [7]. Estrogen binding proteins have been demonstrated in different types of mammary tumors [5, 8, 9].

Using dry-mount autoradiography, which excludes or minimizes translocation and diffusion artifacts, a nuclear concentration of estrogen has been demonstrated in cells of the ovary , uterus, vagina, oviduct, pituitary and brain [7, 10, 11, 12]. In the present study this technique was applied to identify estrogen target cells in mammary glands of lactating and pregnant animals and uteri of lactating animals.

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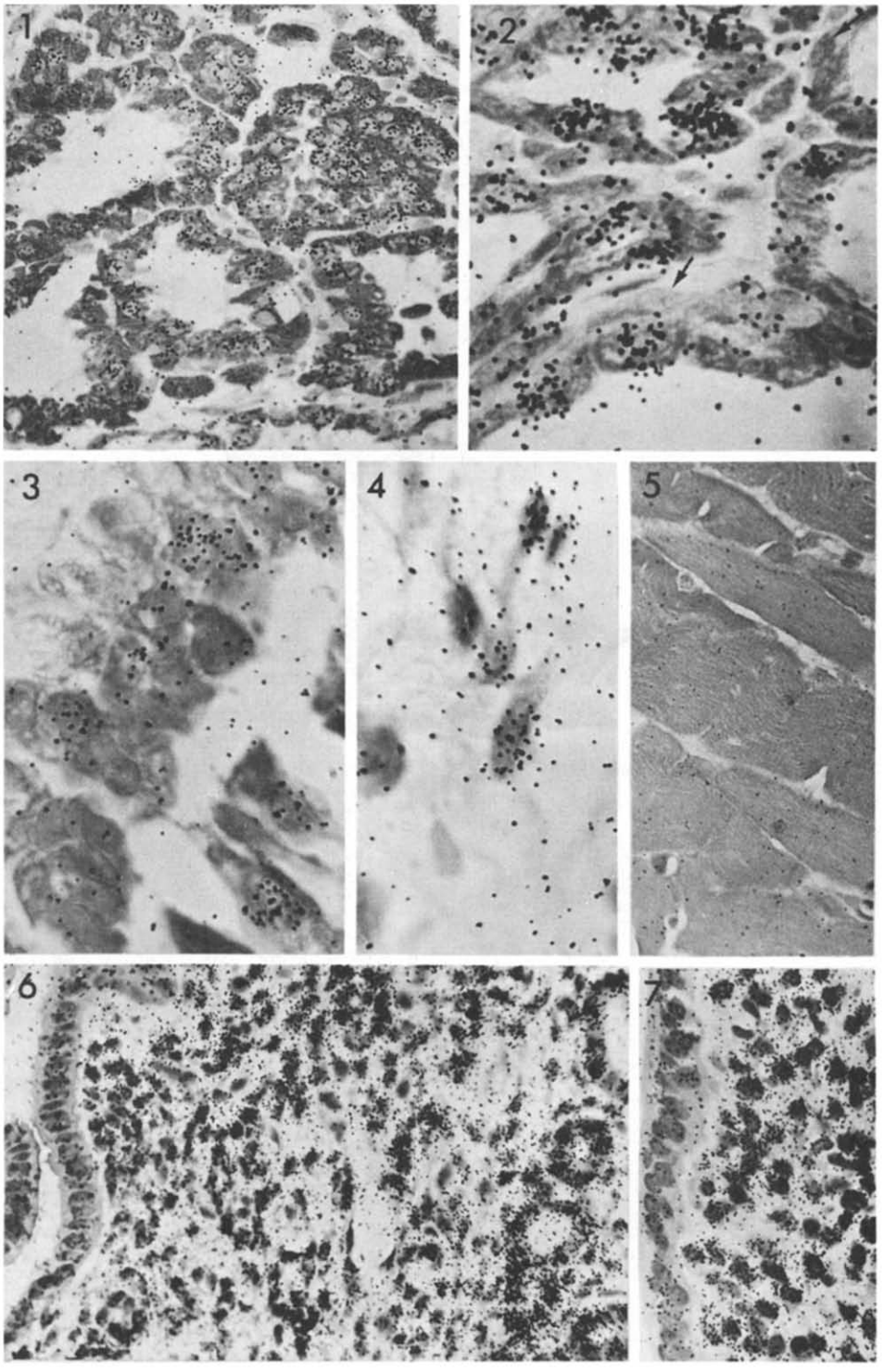
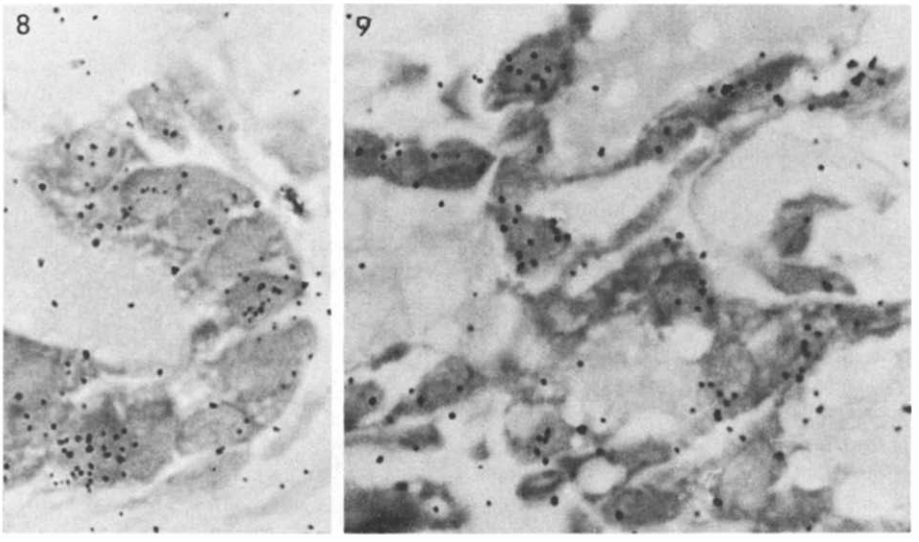
Mammary glands from lactating and pregnant animals were considered in our studies, since their well developed alveoli contain more epithelial cells and less adipose tissue than those from cyclic animals.

MATERIALS AND METHODS

Four female Swiss albino mice (one on postpartum day 11 and three on postpartum day 14), 45–50 g body weight, and two female Sprague–Dawley rats (on postpartum day 21), two pregnant rats (one on day 7 and one on day 13, overiectomized for 24 h), 300 g body weight, were used. [2,4,6,7-^{3}H]-Estradiol-17β, S.A. 95 Ci/mmol, was obtained from New England Nuclear Corp., dissolved in 10% ethanol-isotonic saline, and injected subcutaneously. Mice received 0.5 μg per 100 g body weight, lactating rats 0.3 μg per 100 g body weight, and pregnant rats 0.2 μg per 100 g body weight, of [^{3}H]-estradiol-17β. Doses of estradiol above physiological range were used since lactating animals were not ovariectomized and a relatively large pool of non-radioactive endogenous estradiol existed. One hour after the injection, the animals were killed by decapitation and the right inguinal mammary gland was rapidly dissected. Only one-hour interval was considered in these experiments because at this time nuclear uptake of radioactivity in mammary tissues is known to be maximal [13]. One to two mm^3 pieces of mammary tissues were excised, placed on tissue holders, and frozen in liquified propane at -180°C. Two-micron frozen sections were cut in a Wide-Range Cryostat (Harris Mfg. Corp., North Billerica, Mass.) and freeze-dried in a Cryopump (Thermovac Industries Corp., Copiague, N.Y.).



Figs. 1–7. Autoradiograms of mammary glands, alveoli (Figs. 1, 2), intertubular duct (Fig. 3), connective tissue (Fig. 4), diaphragm (Fig. 5) and uterus (Figs. 6, 7) prepared 1 h after subcutaneous injection of [^{3}H]-estradiol-17β into lactating mouse (Figs. 1 and 6) and rat (Figs. 2–5 and 7). Two micron sections. Exposure time: 210 days. Stained with methylgreen-pyronin. ×480 (Figs. 1 and 5–7); ×1200 (Figs. 2–4). Note the nuclear concentration of radioactivity in epithelial cells (Figs. 1 and 2), basal cells of ducts (Fig. 3), connective tissue cells (Fig. 4), and the absence of labeling in myoepithelial cells (Fig. 2, arrow). Muscle of diaphragm (Fig. 5), a non-target tissue, is not labeled. Figs. 6 and 7 show the lack of labeling in uterine luminal epithelium, in contrast to the labeling of stromal and glandular cells of the uterus.



Figs. 8–9. Autoradiograms of mammary glands from pregnant rats on day 7 (Fig. 8) and 13 (Fig. 9), castrated for 24 h, showing a nuclear concentration of radioactivity in epithelial cells 1 h after subcutaneous injection of [^{3}H]-estradiol-17β. Two micron sections. Exposure time: 175 days. Stained with methylgreen-pyronin. × 1260. Note the weaker labeling of epithelial cells when compared with the labeled cells of the lactating rats (Fig. 2).

The freeze-dried, unfixed and unembedded sections were dry-mounted on desiccated photographic emulsion (Kodak NTB-3) coated slides for autoradiographic exposure. After exposure of 6 to 9 months at −15°C, the slides were developed, fixed and stained with methylgreen-pyronin. Autoradiograms were prepared similarly for uterus, a known target tissue, and for diaphragm, a "non-target" tissue. The technique of dry-mount autoradiography has been described in detail [14].

RESULTS

In the mammary glands of all lactating animals studied, concentration of radioactivity was observed in nuclei of epithelial cells of alveoli and ducts with relatively little radioactivity in the cytoplasm (Figs. 1–3). In the ducts with a double-layered epithelium, basal cells were labeled while luminal cells concentrated little or no radioactivity (Fig. 3). In the labeled cells the ratio of nuclear to cytoplasmic radioactivity was approximately 8 to 1. Some of the ductal and acinar cells were unlabeled. Labeling was not confined to a particular type of epithelial cell, either secretory or non-secretory, cuboidal or columnar. Nuclear concentration of radioactivity was also observed in some of the cells of the surrounding connective tissue (Fig. 4). Myoepithelial cells (Fig. 2) and fat storage cells did not show concentration of radioactivity. The cells of the diaphragm, a non-target tissue, did not retain radioactivity in their nuclei (Fig. 5). Autoradiograms of uterine tissues from the lactating mice and rats revealed a characteristic nuclear labeling of radioactivity in stromal, glandular and muscle cells. In contrast, the uterine luminal epithelial cells showed little or no concentration of radioactivity in their nuclei (Figs. 6 and 7).

In the mammary glands of pregnant rats, a weak nuclear concentration of radioactivity was observed in some ductal and alveolar epithelial cells while not in others (Figs. 8 and 9).

DISCUSSION

The present autoradiographic studies demonstrate a retention and concentration of radioactivity in nuclei of certain epithelial cells in the mammary glands of mice and rats, after the injection of [^{3}H]-estradiol-17β. This nuclear concentration of radioactivity is similar to the nuclear concentration of estrogen in other target tissues, such as uterus, oviduct, vagina, ovary, pituitary and brain [7, 10–12]. The autoradiographic study of mammary tissues from pregnant rats also demonstrates a weak nuclear concentration of radioactivity after [^{3}H]-estradiol administration. A similar concentration of estradiol-17β in the epithelium of DMBA-induced mammary tumors in rats has been reported earlier [7]. Using autoradiography, Sander and Attramadal [15] studied estradiol localization in the breast tissue of female rats. They reported that silver grains were localized mainly over the cytoplasm at all intervals after the injection. The difference in the results obtained by these authors and by us may be attributable to the technique used rather than the *in vivo* distribution of the hormone, since their technique apparently did not prevent translocation of the labeled steroid during the tissue preparation for the autoradiograms. The importance

of appropriate autoradiographic techniques for the demonstration of cellular and subcellular distribution of steroid hormones in target tissues has been reviewed and the implications have been discussed in detail [16, 17]. The chemical nature of the radioactivity in the mammary gland in our experiments has not been determined. However, in comparable biochemical uptake studies of [^{3}H]-estradiol in mammary glands of mice and rats, 70 to 97% of the radioactivity retained was "free" estradiol-17β [3, 4, 9, 13].

Our autoradiographic findings are in close agreement with the results obtained by radioassay experiments in which maximal uptake of radioactivity in the mammary gland was observed one hour after the injection of labeled estradiol [3, 4, 13]. In these experiments, the total uptake of estradiol by the mammary tissues was, however, lower than in the uterus and the vagina. This is probably due to the nonhomogeneity of the mammary tissues, which consist of not only epithelial target cells but to a large extent also of fat cells. In *in vivo* studies, nuclei of the lactating mammary gland have also been shown to retain the labeled estradiol, and specific estradiol binding protein has been demonstrated [13]. The nuclear labeling in mammary glands of lactating and pregnant rats has not been compared since fewer pregnant rats were used in the present experiment.

The distribution of radioactivity in rat uterus after [^{3}H]-estradiol injection during lactation is similar to that observed in early pregnancy [18], i.e., with no labeling of luminal epithelium. Similarly, the absence of estrogen binding in the nuclei of luminal epithelial cells has been observed after progesterone pretreatment [18]. Whether progesterone has such a modifying effect on estrogen binding in the mammary gland remains to be investigated.

In the present autoradiographic study, myoepithelial cells did not seem to accumulate radioactivity, although these cells differentiate from mammary epithelial cells [19]. It is probable that these myoepithelial cells do not develop the ability to retain estradiol during their differentiation. Histochemical and morphological differences have been described between the mammary secretory cells and the myoepithelial cells [20, 21].

Previous reports indicate that estrogen stimulates the secretion of pituitary hormones, especially prolactin and GH, which are essential for the growth of the mammary gland. While the present autoradiographic study demonstrates localization of estrogen in nuclei of epithelial cells of the mammary gland in contrast to [^{3}H]-oxytocin which concentrates in cytoplasm of myoepithelial cells [22], it would be interesting to study the cellular and subcellular distribution of the other hormones which are also involved in the regulation of its growth and functions.

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