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IN VIVO UPTAKE OF ESTRADIOL-17β BY THE UTERUS OF THE MATURE RAT

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SUMMARY

The kinetics of [2,4,6,7-^{3}H]-estradiol-17β uptake by mature rat uterus were studied *in vivo* by dry radioautography. Uterine eosinophils showed a rapid uptake of estradiol with a maximum between one and five minutes following injection. Peak levels of estradiol-17β in the nuclei of luminal epithelial, glandular, stromal and muscular cells were reached between five and twenty minutes after injection. Extraction with water removed the labelled estradiol-17β from all these uterine cell types but not from eosinophils. This property provides further evidence for a distinct estrogen binding system in the uterine eosinophils.

INTRODUCTION

THE STUDY of the interaction of estrogens with their receptor sites may provide information that will contribute to an understanding of the mechanisms of action of these hormones.

Two different binding systems have been postulated in the uterus [1, 2]. The first binding system consists of a cytoplasmic receptor in the 105,000 g supernatant, the 8S receptor [3, 4] and a nuclear 5S receptor [5, 6], together constituting the 8S–5S system [7–9]. The second system involves the uterine eosinophils and *in vitro* studies have shown high affinity, limited binding capacity and a great specificity for compounds with estrogenic activity [1, 2, 10, 11].

The 8S-5S system has been studied exhaustively using different biochemical techniques [3–9]. Radioautographic studies of estrogen uptake *in vivo* by immature rat uterus have shown localization of the steroid in the nuclei of epithelial, gland, stromal and muscle cells [12–14]. However, eosinophils are not present in the uterus of immature rats [11, 15]. *In vivo* localization has not previously been examined in the uterus of mature rats.

In the present report an *in vivo* study of the uptake of estradiol-17β by the uterus of mature rats was done using both freeze-dried sections for radioautography of diffusible compounds, and a differential extraction technique, demonstrating different properties of the 8S–5S and the eosinophil binding systems.

EXPERIMENTAL

[2,4,6,7-^{3}H]-estradiol-17β (95 Ci/mmol) from New England Nuclear Corp. was dissolved in ethanol and diluted in saline to give a final steroid concentration of 5 μg/ml in ethanol–saline 1 : 10.

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Sprague-Dawley rats in the first day of diestrus were maintained under nembutal anesthesia during the experimental procedure. The labelled hormone, at a dosage of 1 μg/100g of body weight, was injected via the jugular vein. In some animals, a dosage of 0·1 μg/100g of body weight was used. Samples of the uterus were taken from each animal through laparotomy at 1, 3, 5, 10, 20, 40, 60 and 120 min after the injection of the isotope. The samples were immediately frozen in liquid propane and then stored in liquid nitrogen. Control samples of skeletal muscle and small intestine were taken at various times.

From each sample, cryostat sections were made and processed in either of the two ways. Some of the sections were freeze-dried and processed using a dry radioautographic method designed for diffusible substances [12]. The remainder of the sections were mounted directly on glass slides and processed by differential extraction. The mounted sections were washed in several changes of distilled water for a total of ten minutes at 20°C, then air dried and coated with a liquid radioautographic emulsion (Kodak NTB-3). After 1, 2, 3 or 10 months of exposure, the radioautograms from both methods were developed in D-19 at 20°C for 30 sec, fixed and stained in hexatoxylin-eosin or methyl green-pyronine.

RESULTS

Extra nuclear radioactivity in the uterus, which corresponds to the extracellular and/or cytoplasmic localization, reaches a maximum within 1 min. after the injection of the labelled estradiol-17β. At this time, the extra nuclear silver grains are randomly distributed (Figs. 2A and B). After 1 min, the extra nuclear radioactivity decreases continuously, and is essentially gone in less than 1 h. Upon close examination of the extra nuclear silver grains in samples taken at 5 min or later, they are found to be non-randomly distributed and tend to be located upon the faintly stained cytoplasm of the stromal cells.

Glandular, stromal and muscular cells appear to follow a common pattern of uptake of estradiol-17β (Fig. 1). Very little concentration of radioactivity over the nuclei of the connective tissue cells is observed within one minute after injection. However, in the vicinity of the blood vessels, there is a tendency for localization of radioactivity in the stromal cell nuclei (Figs. 2A and B). At 3 min after the injection of the labelled steroid, there is already clear localization in the nuclei of glandular, stromal and muscular cells. The amount of radioactivity increases with time, and reaches its maximum between 5 and 20 min after injection (Fig. 1A). Even at the maximum, not all cells of a given type are fully labelled and some show no radioactivity at all. At twenty minutes after injection, the radioactivity starts falling off slowly in the gland cells, and somewhat more rapidly in the other cells (Fig. 1C). However, 2 h after injection there is still considerable radioactivity remaining (Fig. 1G).

The luminal epithelial cell nuclei in general show very little uptake of labelled estradiol-17β (Figs. 1B, D and F). The radioactivity that does appear reaches its maximum between 5 and 20 min after injection, and falls off fairly rapidly after that.

Uterine eosinophils show their maximum uptake between 1 and 5 min after injection (Figs. 2A and B). At this time most of the eosinophils show at least some radioactivity. After this period, the number of silver grains in labelled eosinophils and the number of labelled eosinophils decrease rapidly (Figs. 2C, D and E) and after 1 h very few eosinophils show labelling (Fig. 2F and G). The

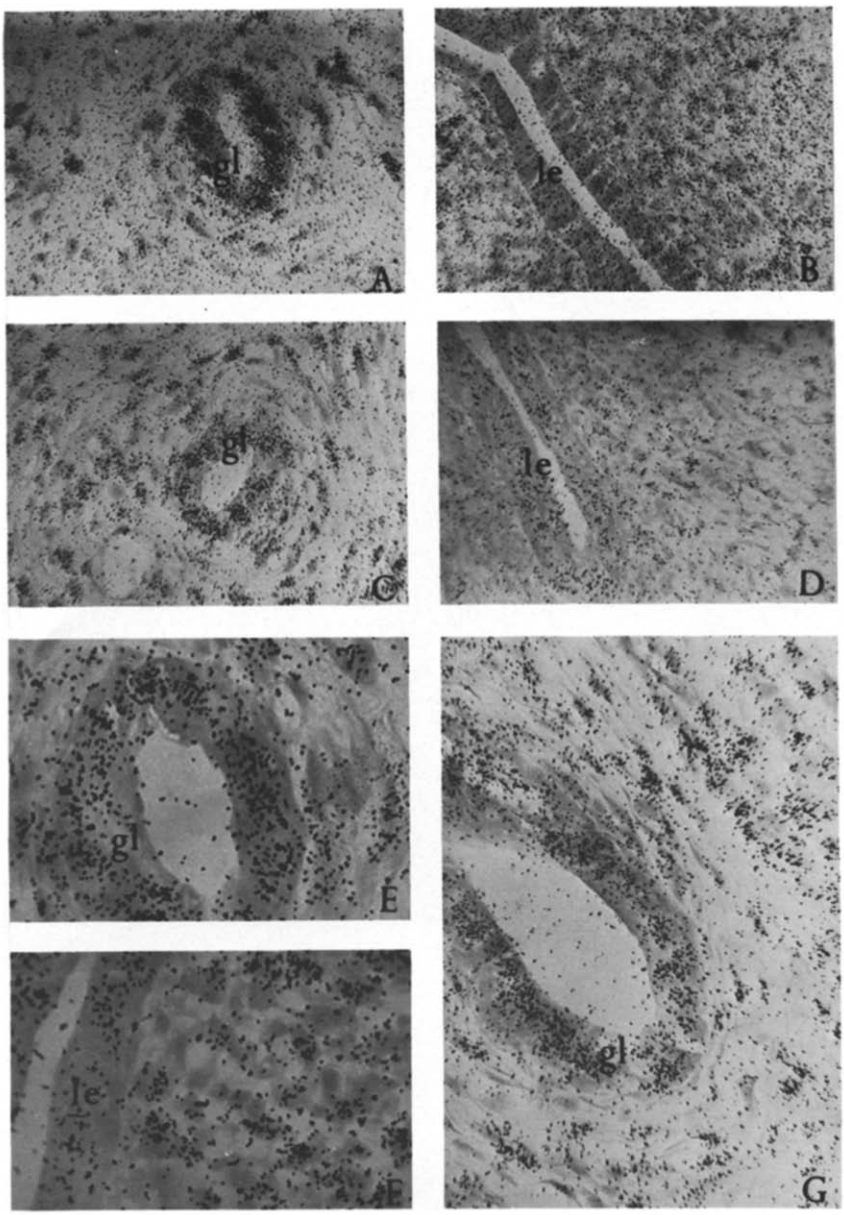

Fig. 1. Radioautograms of [2,4,6,7-^{3}H]-estradiol-17β in luminal epithelial (le), gland (gl) and stromal cells of rat uterus. Samples of uterus were taken 5 min (A, B), 60 min (C, D, E, F) and 120 min (G) after intravenous injection of 1 μg of the labelled hormone per 100 g of body weight. Dry mounted radioautograms of freeze-dried sections were developed after 30 days of exposure and stained with methyl green-pyronine. Magnification $\times$400 (A, B, C, D), $\times$1000 (E, F), $\times$650 (G).

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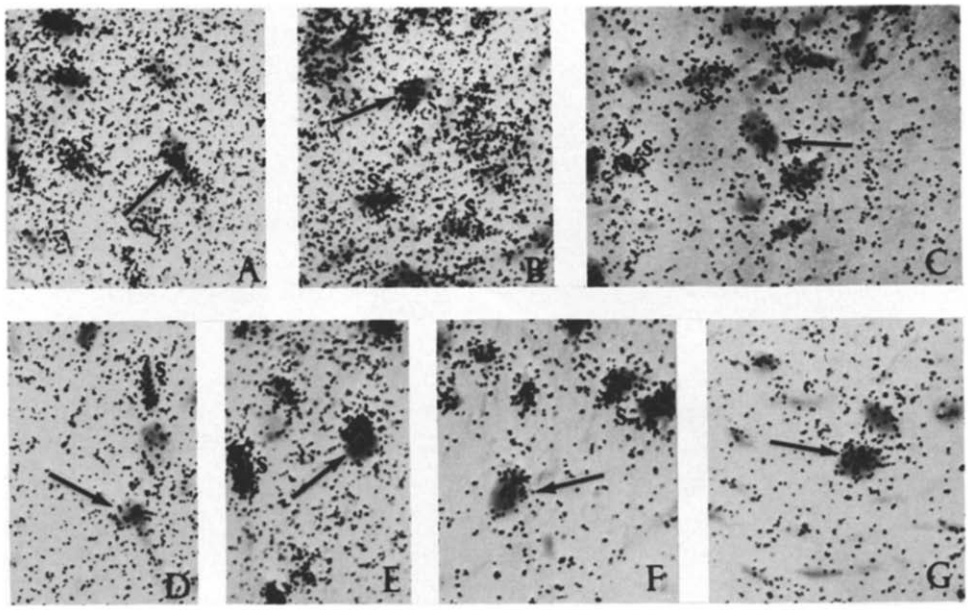

Fig. 2. Radioautograms of tritiated estradiol-17β in rat uterus showing radioactivity in stromal cells (s) and in uterine eosinophils (arrow). Samples of uterus were taken 1 min (A,B), 10 min (C,D,E) and 120 min (F,G) after intravenous injection of 1 μg of the labelled hormone per 100 g of body weight. Dry mounted radioautograms of freeze-dried sections were developed after 90 days of exposure, and stained with hexatoxylin-eosin. Magnification $\times$ 100.

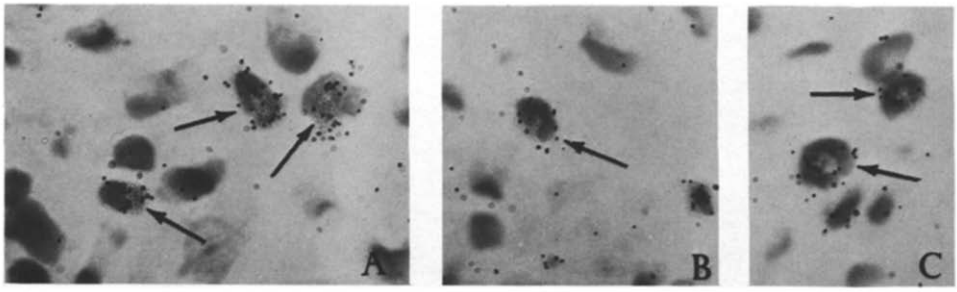

Fig. 3. Radioautograms of tritiated estradiol-17β in uterine eosinophils (arrows). Samples of uterus were taken 5 min, (A) and 20 min (B,C) after intravenous injection of 1 μg of the labelled hormone per 100 g of body weight. Uterine sections were processed by differential extraction (see Experimental) and radioautograms were developed after 60 days of exposure, and stained with hematoxylin-eosin. Magnification $\times$ 1000.

total amount of label in any eosinophil is never higher than an equivalent area of the nuclei of other labelled uterine cell types, and in a large percentage of the eosinophils, the radioactivity is not much higher than that of the extranuclear radioactivity. However, even in the slightly labelled eosinophils, there is a pronounced tendency for the label to concentrate in small heavily labelled areas, causing clumps of silver grains (Figs. 2C and D). These areas tend to be frequently in the cytoplasm near the nuclear membrane. There is also a tendency for grains to appear along small curved lines, perhaps following cytoplasmic interphases (Figs. 2C and 3B).

The control tissues, small intestine and skeletal muscle, show very few silver grains without specific localization at any time that a sample was taken.

Differential extraction of the mounted uterine sections with distilled water removed most of the nuclear radioactivity from all uterine cell types and most of the extra nuclear radioactivity, without removing appreciable quantities of the labelled hormone from the eosinophils (Fig. 3).

The distribution of labelled estradiol-17β was similar in the uteri of animals receiving 0·1 and 1·0 μg per 100 g body weight. However, it was necessary to expose the radioautograms prepared from animals which received the lower dose for 10 months to produce a pattern comparable to that observed after 1 month of exposure in animals which received the higher dose.

DISCUSSION

The kinetics of the *in vivo* uptake of [2,4,6,7-^{3}H]-estradiol-17β by the rat uterus were studied by dry radioautography in different cell types. High levels of radioactivity were found in the nuclei of most uterine cell types, in the extra nuclear space and in the eosinophils shortly after the intravenous injection of the labelled hormone.

Peak levels of estradiol-17β in the nuclei of luminal epithelial, glandular, stromal and muscular cells were reached between 5 and 20 min after the intravenous injection of the labelled hormone. At variance with these results, it was previously reported that the nuclear radioactivity in the uterus of the immature rats was apparent 15 min after the injection of the hormone and that the maximum nuclear concentration was found at the end of 1 h[14]. However, in these studies the hormone was injected subcutaneously and probably the resorption was slow and irregular.

In the present work, using mature rats in the first day of diestrus, low uptake of the labelled estradiol was found in the nuclei of the luminal epithelium, in contrast to the nuclei of other cell types. Previous studies demonstrated high uptake of radioactivity by the nuclei of all the uterine cell types[13].However, this previously reported study utilized immature or ovariectomized mature rats [13], indicating to us that the discrepancy of results is due to different hormonal conditions. Our data suggest that the binding capacity and/or affinity of the luminal epithelium decrease in mature animals in diestrus. This type of experimental study may reveal different responses of the individual uterine cell types under varying hormonal conditions.

In the present work, the dry radioautographic technique demonstrates *in vivo* estrogen localization in the uterine eosinophils, in the nuclei of most of uterine cell types and also in the extra-nuclear space. In previously reported *in vitro* experiments the uptake of estradiol was found only in the eosinophils with essen-

tially no labeling in other uterine cells [1, 2, 10, 11]. It was necessary to reconcile these differences. In the course of the *in vitro* experiments at 20 or 37°C, the sections were subjected to a water extraction step to remove the unbound labelled estradiol [1, 2, 10, 11]. We considered the possibility that the other uterine components had been taking up estradiol during the *in vitro* experiments, but the extraction was removing all the radioactivity except that of the eosinophils. Therefore, we subjected some sections of the present *in vivo* experiments to a similar extraction with water at 20°C for 10 min. This procedure extracted most of the extranuclear and nuclear radioactivity without removing appreciable quantities of the radioactivity from eosinophils. Presumably, the free estradiol-17β, the non-specifically bound estradiol-17β and the radioactivity from the 8S–5S system have been removed by this extraction.

This result implies that the eosinophilic estrogen binding site previously described *in vitro* may also exist *in vivo* under physiological conditions. It is known that uterine eosinophils do not exist in immature or long term ovariectomized animals and that their number is a function of the estrogen level of the animal [11, 15]. Uterine eosinophils undergo ultrastructural modifications in response to estrogenic stimulation [16, 17]. We previously suggested that the eosinophils could be involved in some of the estrogenic early responses in the uterus [2]. Although the recent *in vivo* results correlate with previous *in vitro* results, the actual function of the eosinophils in the uterus still remains unknown.

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