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RADIOAUTOGRAPHIC STUDY OF HUMAN ENDOMETRIUM SUPERFUSED WITH ESTRADIOL-17β, ESTRONE, ESTRIOL AND PROGESTERONE

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SUMMARY

Radioautographic analysis of slices of human endometrium superfused with either tritiated estradiol, estrone, estriol, or progesterone in buffer solutions demonstrated uptake of radioactivity by glandular and luminal epithelial cells, as well as by stroma. The labeled compounds were retained by these cells during prolonged washing with buffer or bovine serum albumin. A partial removal of extranuclear steroids was accomplished by washing of the labeled slices with human male serum.

Nuclear concentration of radioactivity in the labeled cells was already evident after 5 min of incubation. Uniform distribution of radioactivity across slices, approximately 200 μm thick, was achieved only after 20–40 min of perfusion.

Eosinophils showed cytoplasmic labeling during perfusion of radioactive estrone, estradiol and estriol, but were not labeled by progesterone.

Chromatographic analysis of the labeled steroids extracted from the washed tissue slices revealed the presence of both tritiated estrone and estradiol when either of these tracers were superfused.

INTRODUCTION

RADIOAUTOGRAPHIC studies of the uptake of estradiol (E_2†), estrone (E_1), estriol (E_3) and progesterone (P) by human endometrium were undertaken to determine the cytologic distribution of these hormones. The purpose of these studies was to investigate the possibility that receptors for estrogens and progesterone might be preferentially located in a particular type of endometrial cell. Isotope concentrations and nuclear-extranuclear distribution of the radioactivity in stromal and epithelial cells can be expected to yield information about cell type specificity if the identity of the intracellular labeled compounds is known.

The labeling was achieved by superfusion of slices of human endometrial curettings with solutions of tritiated E_1, E_2, E_3 or P in buffer under conditions similar to those selected for experiments in which rates of uptake and metabolism of these hormones were measured by tracer methods[1,2]. A dry radioautographic technique, which minimizes the translocation of the labeled steroids during

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†Abbreviations used: E_2, Estradiol-17β; E_1, Estrone; E_3, Estriol; P, Progesterone; KRBG, Krebs–Ringer bicarbonate buffer; HS, human male serum; BSA, bovine serum albumin.

processing[3], was applied. This technique has been successfully used for the investigation of the distribution of E_2 in rat uteri after *in vivo* labeling[4, 5].

EXPERIMENTAL

Endometrial curettings were sliced with a tissue sectioner (Sorvall TC-2), set at 200 μm, and placed in a superfusion apparatus described elsewhere[1, 6]. A solution of tritiated steroids in Krebs–Ringer bicarbonate buffer, saturated with 95% O_2–5% CO_2, and containing 1 mg/ml of glucose (KRBG) was superfused over the tissue slices at a rate of 18 ml/h for 60 or 120 min at 37°C. At this time, the perfusion solution was replaced by a washing medium from which the labeled steroids were excluded and the superfusion was continued at the same rate for another 60 min. The washing medium was either KRBG buffer, undiluted human male serum (HS) or a solution of bovine serum albumin (BSA) in KRBG (40 mg/ml). Table 1 describes the perfusion data corresponding to each experiment. Samples of tissue were taken at various intervals during the labeling and washing periods. The samples were immediately frozen in liquid propane and stored in liquid nitrogen. From each sample, 4 μm cryostat sections were made and processed in either of two ways. Some of the sections were freeze-dried and submitted to the dry radioautographic technique[3]. The remainder of the sections were thoroughly washed with water before coating with the liquid radioautographic emulsion NTB 3 (Kodak Co., Rochester, N.Y.), following procedures previously described[5]. After 1, 3 or 10 months of exposure, the radioautograms were developed in D-19 solution (Kodak Co., Rochester, N.Y.) at 20°C for 30 s, fixed and stained with hematoxylin-eosin or with methyl-green pyronine.

In order to identify the labeled compounds, the tissue remaining in the superfusion chamber at the end of the labeling and washing period was removed and homogenized in a methanol solution of steroid carriers. After centrifugation, the supernatant was submitted to chromatographic analysis, as described elsewhere[1]. The total amount of a labeled steroid present in the homogenate was estimated by measuring the radioactivity in the purified compound and the recovery of the added carrier. The pellets obtained after centrifugation were used to determine the protein content of the homogenate by the method of Lowry *et al.*[7]. The concentrations of labeled steroids could then be expressed in terms of protein wt. or wt. of wet tissue[1].

Tracers

[6, 7-^{3}H]-E_2 (S.A. 48 Ci/mmol), [2, 4, 6, 7-^{3}H]-E_2 (S.A. 90 Ci/mmol), [6, 7-^{3}H]-E_1 (S.A. 48 Ci/mmol), 6, 7-^{3}H-E_3 (S.A. 50 Ci/mmol), and 1, 2-^{3}H progesterone (S.A. 50·3 Ci/mmol) were purchased from New England Nuclear Corp., and purified by paper chromatography.

All measurements of radioactivity were performed with a liquid scintillation counter (Beckman LS-200) using a toluene solution of POP (2, 5-diphenyloxazole, 0·4%) and POPOP (1, 4-bis 2-(5-phenyloxazolyl) benzene, 0·05%). The counting efficiency was 46%.

The concentrations of tracers in the superfusion medium are shown in Table 1.

RESULTS AND DISCUSSION

(A) *Estradiol superfusion*

(a) *Localization.* Radioautograms of samples of tissue, taken at different times

Table 1. Labeling conditions

Exp #	Type of endometrium	Labeling Medium	Labeling Time (min)	Washing Medium	Washing Time (min)	Concentrations of tritiated steroids: Perfusion medium (million c.p.m./ml)	Washed tissue (million c.p.m./g) E_1	E_2	P
1.	Late Secretory	KRBG	125	KRBG	60	E_2:0·09 (0·5 ng/ml)	0·85	0·065	—
2.	Late Secretory	KRBG	125	KRBG	60	E_2:0·80 (4·4 ng/ml)	6·7	0·17	—
3.	Late Secretory	KRBG	120	KRBG	60	E_1:0·54 (3 ng/ml)	2·9	0·37	—
4.	Late Proliferative	KRBG	120	KRBG	60	P:0·70 (4·7 ng/ml)	—	—	3·3
5.	Secretory (Day 21–23)	KRBG	75	KRBG	60				
				HS	60	E_2:0·84 (2·0 ng/ml)			
				BSA	60				
6.	Early Proliferative	KRBG	60	KRBG	60				
				HS	60	E_3:0·68 (3·8 ng/ml)			
				BSA	60				
7.	Mid-Proliferative	KRBG	60	KRBG	60				
				HS	60	E_2:0·84 (2·0 ng/ml)			
				BSA	60				
8.	Early Proliferative	KRBG	60	KRBG	60				
				HS	60	E_2:0·84 (2·0 ng/ml)			
				BSA	60				

during perfusion and washing, showed labeling of both epithelial and stromal cells (Figs. 1A, B and C, experiment 7), with a distinct nuclear concentration of radioactivity, as previously observed in the rat[5]. The uptake of radioactivity by the luminal epithelium was somewhat lower than that observed in glandular epithelium. Nuclear concentration of radioactivity in cells was noted already at 5 min of perfusion. Eosinophils showed cytoplasmic labeling, as it has already been reported to occur in the rat[5, 8] (Fig. 1C).

No qualitative difference in the distribution of radioactivity was noted in endometrium obtained at different phases of the menstrual cycle, or in the samples perfused with a high or a low concentration of the hormone. However, the length of exposure necessary to obtain the same level of intensity in the radioautograms was much longer in the samples perfused with low concentrations of ^{3}H-E_2 (10 and 1 month for experiments 1 and 2, respectively).

Washing of the tissue slices by superfusion with buffer or BSA did not produce a noticeable reduction in the nuclear concentration or distribution of the radioactivity. Under the same conditions, human male serum removed a significant fraction of the extranuclear radioactivity (experiment 8, Fig. 2).

The results obtained by *in vitro* labeling with ^{3}H-E_2 were very similar to those previously seen in immature[4] and mature[5] rats.

(b) *Penetration of the labeled steroids into the tissue slices.* At the end of the first minute of perfusion there was radioactivity only near the surface of the tissue and no nuclear localization was observed. After 5 min of perfusion, a clear nuclear localization and a deeper penetration of the tracer was noted. However, at 20 min of incubation there still remained a concentration gradient between the periphery and the core of the tissue (approx. 100 μm). The distribution of radioactivity throughout the slices became uniform between 20 and 40 min of perfusion.

(c) *Washing of the labeled compounds from cryostat sections.* When the cryostat sections were washed with water, BSA or serum, the radioactivity was removed from all cells except eosinophils (Fig. 1D).

(d) *Identification of labeled intracellular compounds.* Intracellular concentrations of labeled steroids remaining in some of the tissues after the washing period are listed in Table 1. More E_1 than E_2 was found in tissue during perfusion of ^{3}H–E_2, in agreement with previously reported results[1, 2, 6].

(B) *Estrone superfusion*

The distribution of radioactivity in the various types of cells, including eosinophils, and the penetration of the tracer into the tissue slices showed the same pattern observed during perfusions of ^{3}H–E_2.

As is evident from the results of experiment 3 (Table 1), a significant fraction of perfused E_1 appears as intracellular E_2. Thus, perfusion of either labeled E_1 or E_2 results in a mixture of both compounds in the cells.

(C) *Estriol superfusion*

No differences were noted in the distribution of radioactivity when either tritiated E_1, E_2, or E_3 were superfused. Results from experiments in which labeled E_1, E_2 and E_3 were isolated from nuclei obtained from superfused endometrial slices indicated that E_3 remains practically unchanged and is tightly bound (L. Tseng and E. Gurpide, unpublished observations).

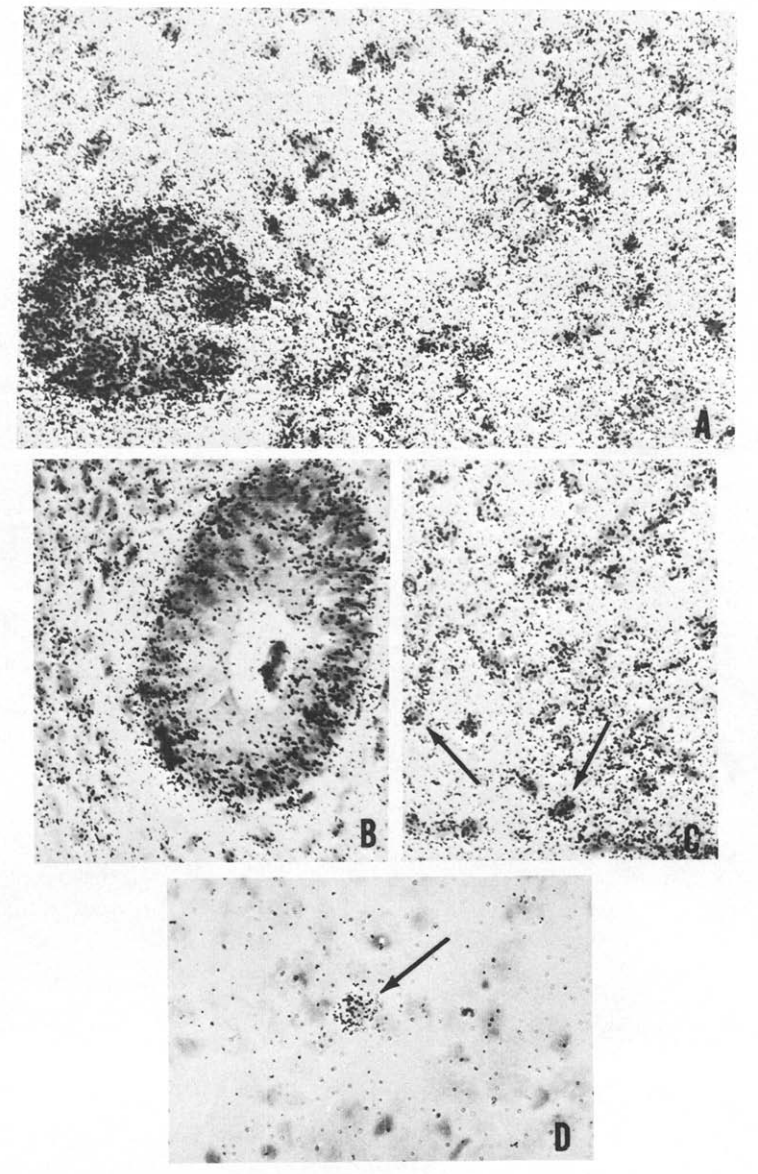

Fig. 1. Radioautograms of sections from slices of mid-proliferative human endometrium taken after 60 min of superfusion with tritiated estradiol-17β (experiment 7). Dry mounted radioautograms of freeze-dried sections were developed after 30 (B and C) or 90 (A) days of exposure. D represents a radioautogram of a cryostat section extracted with water and developed after 60 days of exposure. Arrows point to eosinophils. All radioautograms were stained with hematoxylin-eosin. Magnification 600.

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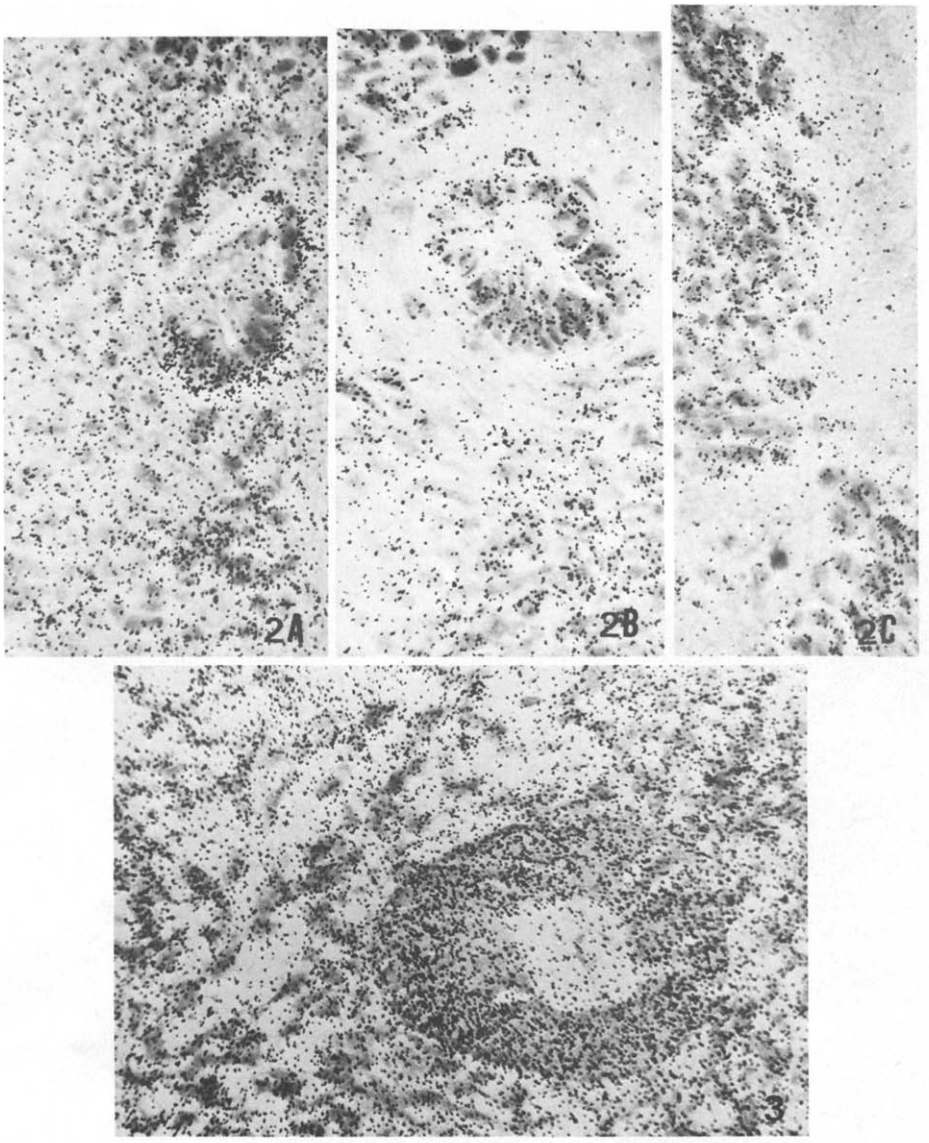

Fig. 2. Radioautograms of sections from slices of early proliferative human endometrium superfused with tritiated estradiol-17β for 60 min and subsequently washed with human male serum (experiment 8). Samples were taken at the end of the superfusion with the tracer (A) or at the end of the washing with human male serum (B and C). Dry mounted radioautograms of freeze-dried sections were developed after 30 days of exposure and stained with hematoxylin-eosin. Magnification ×600.

Fig. 3. Radioautograms of a section from slices of late proliferative human endometrium (experiment 4) taken after 120 min of superfusion with tritiated progesterone and 45 min of wash in KRBG. Dry mounted radioautograms of freeze-dried sections were developed after 30 days of exposure and stained with methyl green-pyronine. Magnification ×800.

(D) *Progesterone superfusion*

Similarly to the results obtained during perfusion of tritiated estrogens, stromal and epithelial cells were labeled approximately to the same extent with perfused P. Figure 3, which corresponds to a sample from experiment 4, is representative of the pattern noted in other samples of tissue obtained during the same experiment. Nuclear concentration of radioactivity in the glandular epithelium and stroma is apparent. Eosinophils were not labeled. As shown in Table 1, P constitutes the bulk of the radioactivity present in the tissue.

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