Kallikrein excretion (Table II) was higher in all groups in the 3rd week than it had been in the 2nd, but in both instances the quantities voided did not differ among the groups.

PRA was greatly depressed in all 3 groups, doubtless because of the salt excess to which they were exposed. All of the control values fell below 0.1 ng/ml/h (Table II), but both steroid-treated groups averaged slightly higher because several individual values ranged 0.1–1.2 ng/ml/h (Table II).

If 16β-OH-DHEA has 1/40th and DOCA 1/25th (the generally accepted figure) the potency of aldosterone, then 2 mg of 16β-OH-DHEA should be equivalent to 1.25 mg of DOCA, which is 6^7 to 10^{17} times the quantity needed to cause hypertension in the rat. The failure of that dosage of 16β-OH-DHEA to have an effect on saline intake, prominently enhanced by DOCA under similar circumstances[18], blood pressure, heart and kidney weight, or kallikrein excretion, casts serious doubt upon its mineralocorticoid status.

In that context FUNDER et al.[19] find that neither renal mc (aldosterone) receptors, nor those responsive to estrogen – a secondary renal sodium-retrieval system – display significant binding affinity for 16β-OH-DHEA. Such binding is considered to be an obligatory prerequisite for mc action, correlating exceedingly well with mc hormone activity. Furthermore, neither EDELMAN et al. (personal communication) using concentrations as high as 5×10^{-6} *M*, nor HIGGINS (personal communication) with concentrations up to 7×10^{-5} *M*, have detected any effect of 16β-OH-DHEA on sodium transport of isolated toad bladders, a generally accepted mc bioassay in which aldosterone exerts maximal stimulation at 4×10^{-8} *M*.

Finally, one of our laboratories (at Dallas) has been unable to demonstrate an effect of 16β-OH-DHEA on the urinary Na/K ratio of adrenalectomized rats at dosages as high as 30 μg, whereas 10 ng of aldosterone, and proportionately larger quantities of the weaker mc's are readily detected in parallel experiments.

These findings do not support the candidacy of 16β-OH-DHEA either as a mc or as a steroid capable of causing hypertension. The significance of its abundance in urine of patients with low renin essential hypertension is thus obscure, and of doubtful etiologic importance. It could be a metabolite of a more active steroid causative of hypertension, or, alternatively, other as yet unidentified steroids may be responsible.

[18] C. E. HALL, S. AYACHI and O. HALL, Tex. Rep. Biol. Med. *30*, 143 (1972).

[19] J. W. FUNDER, J. A. ROBINSON, D. FELDMAN and K. N. WYNNE, Proc. 57th Ann. Meet. Endocrine Soc. New York (1975), Abstract No. 8.

Effect of Progesterone on the in vivo Binding of Estrogens by Uterine Cells[1]

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Summary. Progesterone selectively inhibits estradiol uptake by the nuclei of the luminal epithelial cells but not by other uterine cells. This inhibition in estrogen binding parallels the inhibition by progesterone of some estrogenic responses in the luminal epithelial cells only.

Two important estrogen receptor systems have been found to exist in the rat and human uterus: the cytosol-nuclear and the eosinophil receptor systems[2–6]. Each receptor system seems to be involved independently in the mechanisms of estrogen action[2,7,8]. Therefore, it is possible to produce or selectively block the effects mediated by one of the receptor systems without interfering with the estrogenic response mediated by the other receptor system[9,10].

The cytosol-nuclear receptor system exists in the luminal epithelial, glandular epithelial, stromal and muscular cells of the uterus[3]. It does not necessarily follow that the binding of estrogens to the cytosol-nuclear receptor system must behave in the same manner in all uterine cell types under different physiological conditions. If each cell-type plays a different role in uterine physiology, and if it is possible selectively to block (or stimulate) estrogen binding in one cell type, then it would be possible to modify selected parameters of estrogen stimulation. The present report demonstrates that progesterone selectively blocks estrogen binding in luminal epithelial cells, and correlates this finding with a selective blockage of some parameters of estrogen stimulation in this cell type.

Material and methods. Female rats in proestrus, estrus and in the 1st and 2nd days of diestrus were used, as well as rats in the 2nd day of diestrus pretreated i.p. with either 5 mg of progesterone, 0.3 μg of estradiol, or both together 24 h before the injection of the isotope. Tritiated estradiol (500 μCi, corresponding to 1.4 μg) was injected into the jugular vein, and the animals were sacrificed 10 min or 1 h after receiving the injection of ^{3}H-estradiol. The uteri were excised and processed by a dry radioautographic technique for soluble compounds[11], modified

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[2] A. TCHERNITCHIN, Steroids *19*, 575 (1972).

[3] A. TCHERNITCHIN and R. CHANDROSS, J. Steroid Biochem. *4*, 41 (1973).

[4] A. TCHERNITCHIN, L. TSENG, W. E. STUMPF and E. GURPIDE, J. Steroid Biochem. *4*, 451 (1973).

[5] A. TCHERNITCHIN, J. Steroid Biochem. *5*, 481 (1974).

[6] A. TCHERNITCHIN, X. TCHERNITCHIN, P. ROBEL and E. E. BAULIEU, C. r. Acad. Sci., Paris *280* (Serie D), 1477 (1975).

[7] A. TCHERNITCHIN, J. Steroid Biochem. *4*, 277 (1973).

[8] A. TCHERNITCHIN, J. ROORIJCK, X. TCHERNITCHIN, J. VANDENHENDE and P. GALAND, Nature Lond. *248*, 142 (1974).

[9] A. TCHERNITCHIN, X. TCHERNITCHIN and P. GALAND, Experientia *31*, 993 (1975).

[10] A. TCHERNITCHIN, J. ROORYCK, X. TCHERNITCHIN, J. VANDENHENDE and P. GALAND, Molec. cell. Endocr. *2*, 331 (1975).

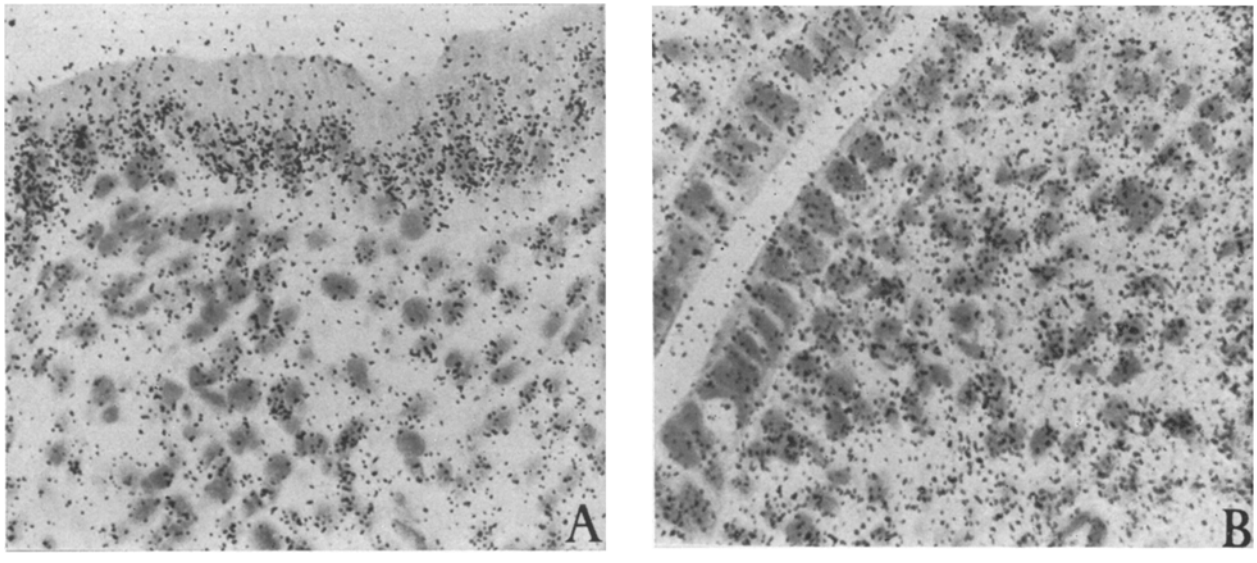
[11] W. E. STUMPF and L. J. ROTH, J. Histochem. Cytochem. *14*, 274 (1966).

Table I. Concentration of tritiated estradiol in rat uterine cells 10 min after the i.v. injection of the isotope

Hormonal condition of the animal	Average of radioautographic granules per								
	Nuclei of luminal epithelial cells	Cytoplasm of luminal epithelial cells	Nuclei of glandular epithelial cells	Cytoplasm of glandular epithelial cells	Nuclei of muscular cells	Nuclei of superficial stromal cells	Nuclei of deep stromal cells	Eosinophils	Extracellular radioactivity
Proestrus	44.3 ± 6.0	16.1 ± 2.3	47.3 ± 6.1	15.1 ± 2.0	34.7 ± 4.2	26.8 ± 3.9	24.7 ± 3.7	16.0 ± 2.2	5.7 ± 0.9
Estrus	43.9 ± 6.0	19.7 ± 2.9	66.4 ± 8.5	24.4 ± 4.0	23.5 ± 3.6	25.3 ± 3.3	24.8 ± 3.4	8.1 ± 1.2	2.8 ± 0.4
1st day of diestrus	11.9 ± 1.2	12.4 ± 1.8	49.2 ± 5.6	20.9 ± 2.4	48.2 ± 6.1	36.4 ± 4.7	40.1 ± 5.8	17.6 ± 2.7	3.8 ± 0.3
2nd day of diestrus	15.6 ± 1.8	16.0 ± 2.0	51.7 ± 7.4	20.9 ± 3.7	31.6 ± 3.6	33.2 ± 4.5	38.5 ± 4.8	6.8 ± 1.7	1.8 ± 0.5
Estrogen pretreated	45.8 ± 5.2	14.8 ± 3.1	65.1 ± 7.2	20.1 ± 2.7	28.2 ± 3.1	28.1 ± 3.9	25.9 ± 4.4	19.4 ± 3.1	3.5 ± 0.6
Progesterone pretreated	12.3 ± 1.8	13.9 ± 1.5	39.8 ± 5.2	14.7 ± 2.2	37.8 ± 5.6	36.4 ± 4.1	47.3 ± 6.2	14.3 ± 2.1	5.4 ± 0.5
Estrogen + progesterone pretreated	12.8 ± 1.3	14.0 ± 1.8	52.1 ± 5.0	21.2 ± 3.1	34.9 ± 3.8	30.2 ± 3.8	41.2 ± 3.1	20.1 ± 3.4	2.7 ± 0.3

Table II. Concentration of tritiated estradiol in rat uterine cells, 1 h after the i.v. injection of the isotope

Hormonal condition of the animal	Average of radioautographic granules per								
	Nuclei of luminal epithelial cells	Cytoplasm of luminal epithelial cells	Nuclei of glandular epithelial cells	Cytoplasm of glandular epithelial cells	Nuclei of muscular cells	Nuclei of superficial stromal cells	Nuclei of deep stromal cells	Eosinophils	Extracellular radioactivity
Proestrus	26.1 ± 3.8	9.8 ± 1.7	34.6 ± 4.2	13.7 ± 2.2	28.1 ± 3.1	19.8 ± 2.8	14.2 ± 2.8	4.6 ± 1.0	0.9 ± 0.2
Estrus	35.2 ± 5.1	11.7 ± 1.8	49.5 ± 6.6	16.2 ± 2.2	16.3 ± 2.2	10.0 ± 2.1	17.4 ± 2.9	6.4 ± 0.9	1.7 ± 0.3
1st day of diestrus	8.1 ± 1.2	6.4 ± 0.9	24.8 ± 3.1	9.9 ± 1.2	26.0 ± 4.1	11.6 ± 2.7	22.8 ± 3.7	4.8 ± 0.7	1.3 ± 0.2
2nd day of diestrus	9.4 ± 1.3	8.7 ± 1.1	36.7 ± 5.1	18.1 ± 2.6	28.4 ± 3.7	26.1 ± 3.7	23.0 ± 3.8	5.4 ± 1.1	0.7 ± 0.1
Estrogen pretreated	34.1 ± 4.1	8.2 ± 1.4	41.7 ± 4.9	14.7 ± 2.0	27.1 ± 3.2	18.7 ± 2.1	14.8 ± 3.0	7.1 ± 0.8	1.0 ± 0.2
Progesterone pretreated	7.5 ± 1.1	6.5 ± 1.0	27.3 ± 3.9	8.7 ± 1.4	25.3 ± 3.7	18.3 ± 2.9	31.4 ± 4.7	3.6 ± 0.7	2.1 ± 0.4
Estrogen + progesterone pretreated	8.2 ± 1.4	8.6 ± 1.0	44.2 ± 3.8	11.2 ± 1.3	27.2 ± 4.1	16.9 ± 3.2	26.3 ± 4.8	6.1 ± 0.8	1.7 ± 0.2



Radioautograms of uterine sections from an estrogen pretreated animal (A) and an estrogen + progesterone pretreated animal (B). The animals were sacrificed 1 h after receiving the injection of ^{3}H-estradiol. The dry radioautograms were developed after 3 months of exposure, and stained with methyl green-pyronine. × 600.

from [12–15]. The radioautograms were developed after 3 months of exposure, and stained with hematoxylin-eosin or methyl green-pyronine.

For each experimental condition and for each cell type, radioautographic granules over 40 cells (and/or nuclei) were counted. The radioactivity of the 'extracellular space' was estimated by counting 40 areas of a size comparable to an average eosinophil, marked by a circle in the ocular piece of the microscope, in areas chosen at random between cells located in the deep stroma.

Results. The uptake of tritiated estradiol by uterine eosinophils in vivo is confirmed in all the hormonal conditions studied in the present investigation (Tables I and II).

The uptake of tritiated estradiol by the nuclei of glandular, stromal and muscular cells is high in all the hormonal conditions investigated (Tables I and II). The uptake of radioactive estradiol by the nuclei of luminal epithelial cells is high in animals in proestrus and in estrus as well as in estrogen pretreated animals, but is very low in animals in the 1st and 2nd days of diestrus, as well as in animals pretreated with progesterone alone or together with estradiol (Tables I and II, Figure).

The nuclear radioactivity in sections of glandular epithelial cells constitutes 65–75% of the total cellular radioactivity (cytoplasmic + nuclear areas) in all the hormonal conditions studied (Tables I and II). Similarly, the nuclear radioactivity in sections of luminal epithelial cells of animals in proestrus and in estrus, as well as in those pretreated with estradiol alone, constitutes 65–75% of the total cellular radioactivity (Tables I and II). In contrast to this, in the animals in the 1st and 2nd days of diestrus, as well as those pretreated with progesterone alone or together with estradiol, the nuclear radioactivity of the luminal epithelial cells constitutes only 50% of the total cellular radioactivity (Tables I and II).

These figures show that progesterone drastically and selectively reduces the uptake of ^{3}H-estradiol by cells of the luminal epithelium. This reduction in binding is associated with obvious morphological changes in this cell type (a reduction in cell size), as observed in the Figure.

Discussion. The progesterone-induced specific inhibition of estradiol uptake by the nuclei of the luminal epithelial cells, but not by other uterine cells, parallels the inhibition by progesterone of some estrogenic responses in the luminal epithelial cells only. The estrogenic responses inhibited in the luminal epithelium are the mitotic activity[16–20], the uridine uptake[21] and the increase in the size of these cells. Since the direct competition of progesterone and estradiol for the receptor sites is unlikely[22, 23], it is probable that progesterone has a specific inhibitory effect on either the synthesis of estrogen receptors or the transfer of the estrogen-cytosol receptor complex from the cytoplasm to the nucleus in the luminal epithelial cells.

[12] I. GERSH, Anat. Rec. *53*, 309 (1932).
[13] I. GERSH, J. VERGARA and G. L. ROSSI, Anat. Rec. *138*, 445 (1960).
[14] I. GERSH, J. biophys. biochem. Cytol. Suppl. *2*, 37 (1956).
[15] I. GERSH, I. ISENBERG, J. L. STEPHENSON and W. BONDAREFF, Anat. Rec. *128*, 91 (1956).
[16] O. I. EPIFANOVA, Abstr. Soviet Med. *5*, 792 (1961).
[17] O. I. EPIFANOVA, Expl. Cell Res. *42*, 562 (1966).
[18] L. MARTIN and C. A. FINN, J. Endocr. *41*, 363 (1968).
[19] F. LEROY, P. GALAND and J. CHRÉTIEN, J. Endocr. *45*, 441 (1969).
[20] J. A. SMITH, L. MARTIN, R. J. B. KING and M. VERTES, Biochem. J. *119*, 773 (1970).
[21] C. TACHI, S. TACHI and H. R. LINDNER, J. Reprod. Fertil. *31*, 59 (1972).
[22] J. ANDERSON, J. H. CLARK and E. J. PECK, Biochem. J. *126*, 561 (1972).
[23] L. TSENG and E. GURPIDE, Endocrinology *93*, 245 (1973).

Peripheral Sympathetic Innervation of the Deep Pineal Gland of the Golden Hamster[1]

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Summary. Both the superficial and deep pineal components of the intact hamster contain a rich network of green to yellow-green fluorescent nerve fibres. After either superior cervical ganglionectomy or after transection of the nervi conarii the majority of the fluorescing fibres disappeared from both the superficial and deep pineal masses. Although the deep pineal remained intact after surgical removal of the superficial pineal, it was devoid of any green or yellow-green fluorescent fibres.

While examining the pineal system of the hamster, SHERIDAN and REITER[3] observed that the pineal is divided into two parts. In addition, to the mass of pineal tissue (superficial pineal) adherent to the under surface of the confluence of sinuses, there is a second pineal mass associated with the habenular commissure (deep pineal). In physiological studies[4, 5], surgical removal of the pineal gland consists of removal of the superficial pineal only. The present study was designed to investigate the peripheral innervation of the deep pineal in intact hamsters and in hamsters that had their superficial pineal removed. For this study the fluorescent histochemical procedure of FALCK-HILLARP[6] for biogenic amine containing neurons was used.

Materials and methods. Groups of anesthesized male hamsters were subjected either to a sham operation, to bilateral superior cervical ganglionectomy[7], to nervi

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[2] Department of Diary Husbandry, University of Missouri, Columbia, Missouri *65201*, USA.
[3] M. N. SHERIDAN and R. J. REITER, J. Morph. *131*, 153 (1970).
[4] R. A. HOFFMAN and R. J. REITER, Science *148*, 1609 (1965).
[5] R. J. REITER, A. Rev. Physiol. *35*, 305 (1973).
[6] A. BJORKLUND, B. FALCK and CH. OWMAN, in *The Thyroid and Biogenic Amines* (Eds. J. E. RALL and I. J. KOPIN; North Holland Publ. Co., Amsterdam 1972), p. 318.
[7] R. J. REITER and R. J. HESTER, Endocrinology *79*, 1168 (1966).