^{3}H-Estradiol Uptake by the Rat Ovary[1] (35960)

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In the hypophysectomized rat, estrogens applied directly to the ovary or injected subcutaneously potentiate the response to exogenous gonadotropins (1, 2). When estrogen alone is used, large doses of estrogen are required to elicit a gross response in the ovary (weight increase). Several tissues in the female rat have been examined for their ability to incorporate ^{3}H-estradiol-17β. The tubular genitalia and mammary gland were shown to be target tissues for estrogen. Studies that used intact (nonovariectomized) rats show that ovarian uptake of estrogen was comparable to tissues that do not respond physiologically to estrogen (3, 4). One drawback of studies concerning ovarian uptake of estrogen is that the ovary itself may be producing sufficient quantities of estrogen—by virtue of the presence of vesicular follicles—to saturate the "estrogen receptors" in the ovary. In order to study the uptake of radioactive estrogen by the ovary, presumably in the absence of endogenous estrogen, hypophysectomized rats were used in the following experiments.

Materials and Methods. Immature, female, Sprague-Dawley rats were used in these experiments. Hypophysectomy or ovariectomy was performed at 21 days and the rats were sacrificed at the age of 27 days. Intact rats of the same age were used as controls when necessary. Estradiol-17β-6,7-^{3}H (40 Ci/m mole; New England Nuclear), 0.1 μg/100 g of body weight, was injected subcutaneously and the rats were sacrificed 2 hr later. The tissues were removed immediately, cleaned, blotted, and weighed. They were either transferred directly to counting vials containing tissue solubilizer (Soluene 100, Packard Instrument Company) or homogenized in 2 ml of water and extracted with a tolune–isoamyl alchohol (19:1) mixture (5). Toluene-based phosphor was added to the samples and tritium was counted in a Nuclear Chicago Scintillation Counter. Each sample was counted for 10 min. and samples were corrected for quenching by use of an internal standard. Results are presented as disintegrations per minute per milligram (dpm/mg) of wet tissue. Student's t test was employed to test the significances of the results.

Results. Uptake of estrogen by the uterus was compared between intact and ovariectomized rats at 27 days of age; this was used as an index of endogenous estrogen activity. Ovariectomy did not affect the concentration of radioactivity in the uterus although the uteri of intact rats were heavier (Table I).

In preliminary experiments, no differences in uptake of radioactivity were noted between the right and left ovary or uterine horn in the animal. Therefore, one ovary and one uterine horn from each animal was solubilized in tissue solubilizer to obtain total radioactivity in the tissue and the remaining ovary and uterine horn were homogenized and extracted to obtain the extractable radioactivity. Muscle and 0.1 ml of plasma were also treated similarly. While there were no differences in ovary and uterus for the two methods of processing, the muscle and plasma contained less extractable radioactivity (Table II). This difference may have been due to the presence of radioactivity in the form of conjugated estrogens. Extractable plasma radioactivity was significantly higher in the hypophysectomized rats than the intact controls, which may be due to slower renal clearance in the hypophysectomized rats. Such differences did not exist in the

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TABLE I. Effect of Ovariectomy on ^{3}H-Estradiol Uptake by the Uterus of 27-Day-Old Rats.

Treatment	No. of rats	Av conc of radioactivity (dpm/mg ± SE)	
		Uterus	Muscle
Ovariectomized	6	214 ± 4.6	6 ± 0.3
Control	6	237 ± 12.8	5 ± 0.4

muscle and uterus. The ovaries of hypophysectomized rats had significantly greater radioactivity than the intact controls. Ovarian and uterine weights were significantly reduced in the hypophysectomized rats. There was a 3-fold difference in the total radioactivity, as well as the weight of the uterus between intact and hypophysectomized rats, but the radioactivity per milligram of tissue did not differ. Total radioactivity in the ovary was not different between the two groups, but the approximately 3-fold difference in weight has significantly changed the results when expressed as concentration.

It was shown previously that pretreatment of rats with cold estrogen or an estrogen antagonist would prevent the uptake of labeled estrogen by the uterus (4, 6). Results are presented in Table III on the effect of pretreatment of hypophysectomized rats with estradiol-17β (Sigma Chemical Company), 10 μg/100 g of body weight, and an estrogen antagonist 1-[2-[*P*-(3,4-dihydro-6-methoxy-2-phenyl-1-napthyl) phenoxy]ethyl]-pyrrolidine, hydrochloride (Upjohn Company), 300 μg/100 g of body weight, 2 hr before ^{3}H-estradiol on the ovary. There was a significant reduction in the uptake of radioactivity by the ovary and uterus while there were no differences in muscle.

Discussion. Autoradiography of the ovary from animals treated with radioactive estrogen provided evidence that the granulosa cell in the follicle is the receptive cell in the ovary (7). In the present study, uptake of labeled estrogen by the ovary was higher in the hypophysectomized rat than the intact but it was not equal to that of the uterus. Pretreatment of rats with cold estrogen or an estrogen antagonist prevented the uptake of labeled estrogen to the same magnitude in

TABLE II. Effect of Hypophysectomy on ^{3}H-Estradiol Uptake by the Ovary and Uterus of the Rat.

Treatment	No. of rats	Ovary[a]		Uterus[a]		Muscle		Plasma (0.1 ml)	
		E	S	E	S	E	S	E	S
Av conc of radioactivity (dpm/mg ± SE)									
Intact	8	67 ± 7.7	68 ± 5.9	284 ± 31.1	320 ± 13.4	4 ± 0.5	7 ± 0.3	147 ± 5.8	1117 ± 169.0
Hypophysectomized	8	154 ± 24.2[b]	148 ± 14.1[b]	288 ± 33.2	257 ± 25.2	4 ± 0.5	7 ± 0.4	166 ± 15.8	2072 ± 273.7[b]
Av organ wt (mg ± SE)									
Intact		8.6 ± 0.7	7.9 ± 0.2	22.8 ± 0.4	24.0 ± 3.3				
Hypophysectomized		3.3 ± 0.5[b]	3.1 ± 0.4[b]	7.9 ± 0.4[b]	7.8 ± 0.4[b]				

[a] One ovary and one uterine horn was processed either by extraction (E); or solubilization (S). Right or left ovary and uterine horn was assigned randomly to E or S method.

[b] Significantly different from control value ($p < .01$).

TABLE III. Effect of Pretreatment on ^{3}H-Estradiol Uptake by the Hypophysectomized Rats.[a]

Treatment	No. of rats	Av conc of radioactivity (dpm/mg ± SE)		
		Ovary	Uterus	Muscle
Control	6	132 ± 8.9	287 ± 20.5	10 ± 1.0
Estradiol-17β	6	21 ± 4.3[b]	27 ± 3.2[b]	8 ± 0.9
Estrogen antagonist	6	65 ± 9.0[b]	140 ± 32.0[b]	12 ± 3.3

[a] Estradiol-17β or antiestrogen was injected 2 hr before ^{3}H-estradiol.
[b] Significantly different from control values ($p < .01$).

the ovary and uterus (about 85 and 50% for estradiol and an estrogen antagonist, respectively). Therefore, uptake of estrogen by the ovary is subject to changes similar to those in the uterus.

The ovary, being a heterogeneous tissue and a source of estrogens, requires a different approach from that of the uterus to study the estrogen receptor sites. The reduction in ovarian weight in the hypophysectomized rats seems primarily due to the loss of large vesicular follicles (8). However, it has not been ascertained whether the capacity to produce estrogen by such an ovary is completely abolished. The fact that the total uptake of estrogen by the ovary of intact and hypophysectomized rats was similar, seems to indicate that the small follicles could be the location of free (unbound) estrogen receptors. It is possible that the estrogen receptors exist in the large follicles as well but they may be saturated with the endogenous estrogen produced by the follicle itself. The variation in the estrogen uptake by a target organ may be explained on the basis of the number of receptive cells per unit of tissue and the availability of free receptors. DeHertogh and co-workers (9) suggested that the uterine uptake of estrogen is dependent on the cell mass which may be responsible for the cyclic variation in the concentration of labeled estrogen. Such may be the case in the ovary also. Cell mass per unit of tissue may be less in the ovary than that in the uterus. This may be true particularly for the estrogen-responsive cells.

Summary. In vivo uptake of ^{3}H-estradiol by the ovary was studied using hypophysectomized, immature, female rats. Radioactivity in the tissues was determined 2 hr after a subcutaneous injection of ^{3}H-estradiol-17β (0.1 μg/100 g of body wt) into 27-day-old rats. Total uptake of radioactivity was similar in the ovaries of hypophysectomized and intact rats but radioactivity per milligram of the ovary in the hypophysectomized rat was double that in the intact. Pretreatment with cold estradiol or an estrogen antagonist prevented uptake of radioactivity to the same magnitude in the ovary and uterus.

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