

Effect of Hypophysectomy and Pretreatment on Uptake and Retention of Estradiol by the Ovary¹ (38137)

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One of the properties of a target tissue is its ability to selectively bind and retain a specific hormone for a considerable length of time (1). Such specific binding and retention of the hormone is associated with its physiological role in the target tissue. Evidence for a physiological role for estradiol in the ovarian follicular development has been reported in the past (2). In a previous report we have demonstrated that in the absence of endogenous estrogen a greater amount of ³H-estradiol-17 β was incorporated by the ovary in hypophysectomized rats (3). In the following studies we have further examined the changes in the uptake and retention of ³H-estradiol by the ovary following hypophysectomy and pretreatment with cold estradiol. The effect of interval following hypophysectomy on the ³H-estradiol uptake by the ovary was also studied.

Materials and Methods. Immature, female, Sprague-Dawley rats were used in these experiments. In one study rats were hypophysectomized at 28 days of age (day 0) and sacrificed at 1, 2, 3, 5, and 7 days following hypophysectomy. Two additional groups of hypophysectomized rats were pretreated with estradiol-17 β (0.1 or 10 μ g) at day 5 and sacrificed 48 hrs later. Intact rats 28 days old were used as controls. All rats received a single subcutaneous injection of estradiol-17 β -6,7-³H (40 Ci/mM, New England Nuclear), 0.1 μ g/100 g body wt 2 hr before sacrifice.

In another study rats were hypophysectomized at 21 days of age. One group of rats received estradiol-17 β (1.0 μ g/100 g body wt per day) from day 23 through day 25 of age. Intact rats, 26 days of age, were used as con-

trols. All rats received estradiol-17 β -6,7-³H (46 Ci/mM), 0.1 μ g/100 g body wt, at 26 days of age and were sacrificed 2, 4 and 8 hr later.

All rats were guillotined and the tissues (ovaries, uteri, and skeletal muscle) were removed immediately, cleaned, blotted and weighed (uteri were slit lengthwise to expel fluid, if any). The tissues were homogenized in 2 ml of water and extracted with a toluene-isoamyl alcohol (19:1) mixture (4). Toluene based phosphor was added to the samples and tritium was counted in a Packard Tricarb Liquid 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 min (DPM). The muscle tissue failed to respond significantly to any of the treatments as has been observed previously (3). Therefore, the data for muscle were deleted from the results presented. Student's *t* test was employed to test the significance of the results.

Results. Effect of interval after hypophysectomy. Ovarian and uterine weights decreased significantly following hypophysectomy (Table I). Pretreatment with cold estradiol (0.1 μ g or 10 μ g) 48 hr prior to sacrifice significantly increased uterine weight at both dose levels when compared to day 7 hypophysectomized rats. Significant increase in ovarian weight occurred only in response to 10 μ g dose. Uptake of radioactivity per ovary increased significantly while the uptake by the uterus decreased following hypophysectomy when compared to the intact controls. Pretreatment with cold estradiol 48 hr before radioactive estradiol did not significantly alter the uptake by the ovary. There was a dramatic increase in the uptake by the uterus

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TABLE I. Effect of Interval after Hypophysectomy and Estrogen Pretreatment on Uptake of ^3H -Estradiol-17 β .^a

Number of rats	Organ	Controls	Days following hypophysectomy					Pretreatment ^b	
			1	2	3	5	7	$E_{0.1}$	E_{30}
Wet wt mg	Ovary	7	18.1 ± 0.9	11.1 ± 0.7	11.1 ± 1.1	7.6 ± 0.3	6.6 ± 0.5	8.2 ± 0.6	8.5 ± 0.4
		7	50.9 ± 5.0	24.1 ± 0.9	21.7 ± 1.0	19.1 ± 0.6	17.4 ± 0.5	31.0 ± 1.1	41.6 ± 1.5
DPM Per organ	Ovary	7	563 ± 62.7	1051 ± 81.2	1279 ± 135.1	983 ± 73.5	843 ± 63.7	903 ± 78.5	1180 ± 115.8
		7	9457 ± 806.7	7807 ± 1121.8	6860 ± 740.0	6743 ± 902.6	6533 ± 505.7	10141 ± 734.9	16276 ± 937.6

Mean ± SE.

^a Hypophysectomy was performed at 28 days of age (day 0). ^3H -Estradiol-17 β was given sc (0.1 $\mu\text{g}/100$ g body wt) and the rats were sacrificed 2 hr later. Intact rats, 28 days of age, were used as controls.

^b Two groups of hypophysectomized rats were given 0.1 μg or 10 $\mu\text{g}/100$ g body wt ($E_{0.1}$, E_{30}) of Estradiol-17 β sc at 5 days after hypophysectomy and used 2 days later (i.e., 7 days following hypophysectomy).

which appears to be dependent upon the dose of estradiol. Concentration of radioactivity (DPM per mg wet issue) increased significantly following hypophysectomy in the ovary as well as the uterus (Fig. 1). The uptake by the ovary increased up to day 3 following hypophysectomy and thereafter there was no change. Pretreatment with 0.1 μg cold estradiol tended to suppress the concentration of radioactivity in both organs but had no appreciable effect when 10 μg of cold estrogen was administered (when compared to day 7 hypophysectomized group).

Uptake and Retention of ^3H -Estradiol by the Ovary and Uterus. The uptake of radioactive estradiol per mg of wet ovary was significantly higher in hypophysectomized rats compared to intact controls (Fig. 2). Pretreatment affected the initial uptake but by 4 and 8 hr the retention was similar to non-treated hypophysectomized rats. The uptake of radioactivity by the uteri of intact and

hypophysectomized rats was similar at 2 hr but uteri of intact rats lost the radioactivity much more rapidly than the hypophysectomized rats. The uptake and retention for pre-treated rat uteri followed a similar pattern as that for ovaries. When the radioactivity was expressed in terms of tissue protein (Fig. 2) the initial uptake was very high in the hypophysectomized rat ovaries and the retention at 8 hr was somewhat similar to that of the uterus. Pretreatment with cold estradiol did suppress the uptake by the ovary but it was significantly higher than the intact controls. When radioactivity was expressed in terms of μg DNA the hypophysectomized rat ovaries showed significantly higher uptake and retention when compared to intact controls (Fig. 2). In the uterus there were no differences between the hypophysectomized and intact rats. Pretreatment of hypophysectomized rats somewhat depressed the uptake and retention in the ovary but greatly enhanced the uptake and retention by the uterus.

Discussion. The above results confirm our previous findings that in the absence of endogenous estrogen the ovaries incorporate greater amounts of radioactive estradiol (3). Jensen *et al.* (1) have estimated that the receptor protein in the ovary was about 2% that of the uterus. Such low values may have been due to the use of intact rats although they were immature. Our results on the effect of interval after hypophysectomy (Fig. 1) show that the ovarian uptake increased progressively with the interval after hypophysectomy. This suggests that the loss of endogenous estrogen is not as abrupt when the animal is subjected to hypophysectomy as one would expect after ovariectomy. Further, we have used rats of two different ages. In the younger rats (Fig. 2) the uptake by the uterus was not different between the intact and hypophysectomized animals whether the uptake was studied on the basis of wet wt or protein or DNA. The uptake by the ovary increased significantly following hypophysectomy. In the older rats (Fig. 1) the uteri of intact controls had much lower uptake when compared to any day following hypophysectomy. Recent reports (5, 6) provided evidence that the ovaries of immature rats are capable of producing estrogen. Estimation of cytoplasmic receptors accounts for only the "free" receptors. There-

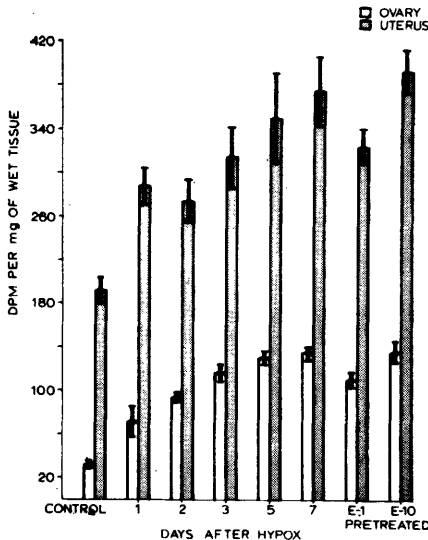


FIG. 1. Effect of interval after hypophysectomy and pretreatment upon the uptake of ^3H -estradiol (DPM/mg wet tissue \pm SE) by the ovary and uterus. Control rats were 28 days of age. Rats were hypophysectomized at 28 days of age and sacrificed 1, 2, 3, 5 and 7 days later. Pretreated rats were given estradiol either .1 μg (E-1) or 10 μg (E-10) per 100 g body wt on day 5 and sacrificed on day 7 following hypophysectomy. All rats received ^3H -estradiol (.1 μg /100 g body wt) 2 hr before sacrifice.

UPTAKE OF ESTRADIOL BY THE OVARY

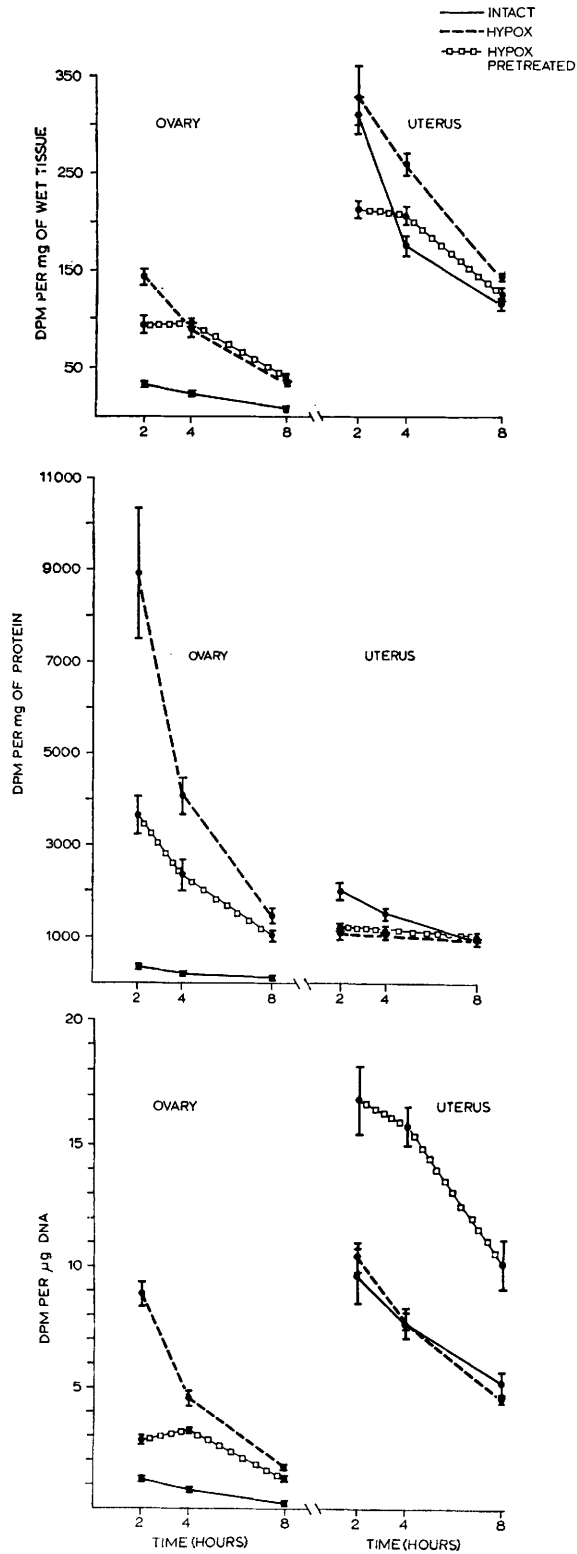


FIG. 2. Uptake and retention of ^3H -estradiol by the ovary and uterus in intact, hypophysectomized and pretreated rats. Radioactivity is expressed as DPM per mg wet tissue (top), per mg protein (middle) and per μg DNA (bottom). Hypophysectomy was performed at 21 days of age and

autopsy was at 26 days. Intact rats were 26 days old. Cold estradiol (pretreatment) was given on days 23 through 25 following hypophysectomy. Rats were sacrificed 2, 4 and 8 hr following a single injection of ^3H -estradiol ($.1 \mu\text{g}/100 \text{ g body wt}$).

fore, one is likely to obtain very low values when working with the ovaries of intact animals.

It was suggested that the quantity of receptor present in any tissue is a function of the cell numbers (7). In spite of the drastic reduction in DNA of the ovary following hypophysectomy (Fig. 2) there was an increase in the uptake of radioactive estradiol. This is possible if more receptors are made available ("free") due to the loss of endogenous estrogen. Also it appears that only a fraction of the whole ovarian tissue is involved in the binding of estrogen.

It has been postulated that the estrogen receptor protein of the uterus may be selectively retained while other protein components are subject to degradation in the absence of endogenous estrogen which may be the cause for uterine atrophy following ovariectomy (8). One could speculate such phenomena occurring in the ovary not only in regard to protein component but the whole cell. The ovary is also dependent on the gonadotropins for its structural integrity. Following hypophysectomy the ovarian components dependent on gonadotropins are likely to degenerate quickly but those dependent on estrogen are retained for a longer period of time. We observed that the DNA content of ovaries of hypophysectomized animals was reduced to $\frac{1}{4}$ of the intact animals but the uterine loss of DNA was only $\frac{1}{2}$. When these animals were pretreated with estrogen the DNA content was completely restored in the uterus but not in the ovary.

Summary. Immature female rats were used

after hypophysectomy to study the characteristics of uptake and retention of ^3H -estradiol- 17β by the ovary. The concentration of radioactivity in the ovary increased progressively up to 3 days following hypophysectomy. In another study uptake and retention of ^3H -estradiol was studied at 2, 4 and 8 hr following a single sc injection ($.1 \mu\text{g}/100 \text{ g body wt}$). The ovaries of hypophysectomized rats took up and retained highest amount of radioactivity. The results demonstrate the ability of the ovary to incorporate exogenous estrogen in the absence of endogenous estrogen. Evidence also indicates that the ovaries of immature rats are capable of secreting estrogen.

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