The Effect of Estrogen Priming on the Uptake of Radioactive Estradiol[1] (34477)

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The action of estrogens on target organs has been amply reviewed (1–3). Since the report by Jensen and Jacobson (4), demonstrating accumulation and retention of estradiol in rat uterine tissue, many investigators have turned to the use of tritiated estradiol with high specific activity as a tool for studying mechanisms of estrogen action. The effect of estrogen pretreatment on the uptake of radioactive estradiol has been studied in several laboratories with variable results (4–8). This report defines the conditions under which various estrogens stimulate the uptake of tracer estradiol by the mouse uterus.

Materials and Methods. Five to ten female Cox mice weighing 11–13 g were used per group. At sacrifice, the uteri were quickly removed, dissected free of extraneous tissue, and weighed after uniform blotting to remove excess fluid. The uteri were transferred directly to counting vials and dissolved in 1.0 ml NCS Reagent (Nuclear Chicago) with gentle warming. Diatol was added and radioactivity was determined by liquid scintillation spectrometry. Disintegrations per minute were determined by using an internal standard.

Estradiol-6,7 ^{3}H (42.5 Ci/mM) was obtained from New England Nuclear. Tracer injection solutions were prepared in 5% ethanol-saline and contained 1 μCi/0.1 ml (6.4 ng estradiol/μCi). In all cases the tracer dose of 1 μCi estradiol was given exactly 1 hr prior to sacrifice. Priming doses of estrogen were prepared in corn oil or saline, and the concentration was adjusted so that the injection volume was 0.1 ml.

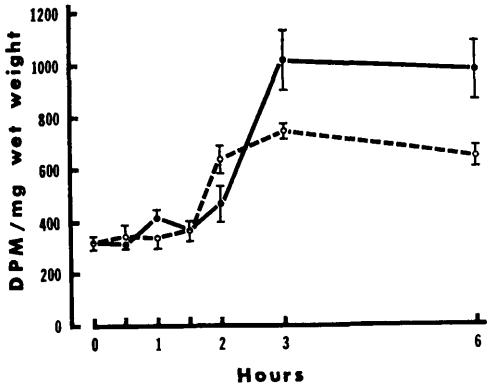

FIG. 1. The effect of 0.03 μg of estradiol-17β injected subcutaneously (solid line) or intravenously (broken line) at various times prior to sacrifice. All animals received 1 μCi (6.4 ng) of 6, 7 ^{3}H-estradiol-17β 1 hr before sacrifice. There were five mice in each group.

Results. When 0.03 μg estradiol-17β was given subcutaneously in corn oil or intravenously in saline at various times up to 6 hr prior to sacrifice, the uptake of tracer estrogen was increased at 3 hr after priming with no further increase at 6 hr (Fig. 1). The response developed somewhat faster with intravenous injection, but the magnitude was not so great.

To study the response for longer periods and to determine the effect of repeated daily doses of estrogen, groups of immature mice were sacrificed at 3, 6, 12, and 24 hr after a priming injection of 0.03 μg of estradiol. The remaining mice were given another priming dose on each successive day and uptake was determined at the designated times. The results show that the priming response repeats daily over a 3-day period, and the effect is

[1] The authors express their thanks to William E. Flanary and Ronald E. Mincy for their technical assistance, and to Jean D. Pieper for the radioactive determinations.

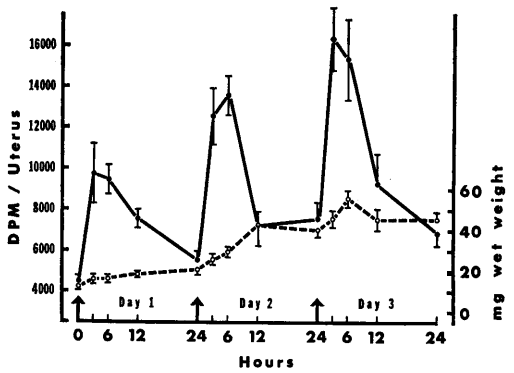

FIG. 2. Uterine uptake of 1 μCi (6.4 ng) of 6, 7 ^{3}H-estradiol-17β (solid line) given 1 hr before sacrifice to animals receiving single or multiple injections of 0.03 μg of estradiol-17β at various times prior to sacrifice. The arrows (↑) represent the points at which priming injections were given. Uterine weight is indicated by the broken line. There were 5 or 10 mice at each point.

largely lost by 12 hr (Fig. 2). Successive priming doses elicit progressively larger responses in terms of total estradiol uptake roughly proportional to uterine weight increase.

The effect of dosage of estradiol-17β used for priming 6 hr before sacrifice is demonstrated in Fig. 3. The response is present with 0.001 μg, and reaches maximum at 0.01 μg, above which a reversal of the effect is seen. A similar response is seen with estriol even though it is a weak uterotropic agent. It was slightly more effective at low doses, but did not produce as much inhibition at high doses. There was no significant difference in maximum response. In ten subsequent experiments estradiol was 1.13 ± .09 (SE) times estriol as a priming agent when given at 0.01 μg.

In order to study the specificity of the priming response, different estrogens were tested. A dose of 0.1 μg of diethylstilbestrol (DES) administered orally or subcutaneously 3 hr before sacrifice increased the uptake of tracer estradiol (Table I). Simultaneous administration of 10 μg of DES significantly inhibited the uptake of estradiol. As a priming agent, mestranol also enhanced the uptake of tracer estradiol at low doses (Table II). As the dose was increased, inhibition of

TABLE I. The Effect of Diethylstilbestrol (DES) on Estradiol Uptake by the Uterus.[a]

Group	Time	dpm/mg	SE
Untreated		395.8	± 51.7
DES 0.1 μg sc	2 hr prior	668.7	± 53.4
DES 0.1 μg oral	2 hr prior	766.0	± 51.7
DES 10.0 μg oral	simultaneously	33.5	± 3.5

[a] There were 10 animals per group and all received 1 μCi (6.4 ng) of 6, 7 ^{3}H-estradiol-17β 1 hr before sacrifice; the time DES was given with respect to the tracer is indicated in the table.

the uptake was observed. A dose-response curve for the priming effect of estradiol-17α is shown in Fig. 4. The dose requirement for this weaker uterotropic agent appears to be about 100 times greater than that of estradiol-17β.

Discussion. The data in this report clearly show an early priming effect of estrogens on the uptake of tracer estradiol. When Jensen and Jacobson (4) prestimulated rats with cold estrogen, the tracer was administered on the day after the final stimulating dose. Our data indicate that the priming effect on uptake is lost after about 12 hr. Similarly, the data of Eisenfeld and Axelrod (5) on uptake in prestimulated rats very likely corresponds to the 24-hr time period, since radioactive estradiol was given 48 hr after the final stimulating dose.

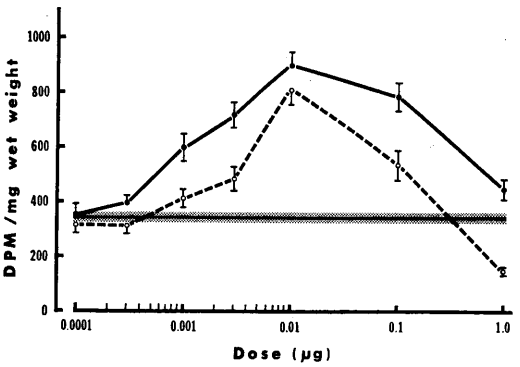

FIG. 3. A comparison of the effect of various doses of estriol (solid line) and estradiol-17β (broken line) on the uptake of 1 μCi (6.4 ng) of 6, 7 ^{3}H-estradiol-17β. All priming doses were given 6 hr before sacrifice. Control is represented by 30 animals (shaded area indicates ± standard error) and other groups contain 10 or 20 animals from combined experiments.

TABLE II. The Dose Response of Mestranol Priming.[a]

Group	Dose (μg)	Uterine dpm/mg	SE
Control	0	245	± 22
Mestranol	0.03	344	± 39
Mestranol	0.3	633	± 43
Mestranol	1.0	606	± 53
Mestranol	3.0	321	± 35
Mestranol	10.0	162	± 31

[a] There were 10 animals per group and all received 1 μCi (6.4 ng) of 6, 7 ^{3}H-estradiol-17β 1 hr before sacrifice. Mestranol was administered subcutaneously 6 hr prior to sacrifice.

Terenius (6) injected 10 μg of estradiol-17β for prestimulation and measured the uptake of radioactive estradiol at 3, 12, 24, and 48 hr. The stimulation of uptake was masked by isotope dilution since 1 μg of estradiol-17β will produce inhibition of the uptake of labeled estrogen. However, when the weak estrogen estradiol-17α was given at 0.1, 1.0, and 10 μg 4 hr prior to sacrifice there was some stimulation of uptake of tritiated estradiol-17β at the two lower doses. An augmentation of uptake is observed with doses up to 1.0 μg of estradiol-17α, above which a reversal of the effect is seen.

It was shown by Terenius (6) that saturation of mouse uterine receptors occurs at about 0.3 μg of estradiol-17β with systemic injection. It is apparent that the priming dose of 0.03 μg is below the saturating dose since there is no depression of uptake at any time. In addition, Terenius demonstrated that the tracer dose of 0.01 μg of estradiol-17β had a definite uterotropic effect if injected into mice for 3 days. It is obvious from the data presented here that, in experiments which extend beyond 2 hr after a physiologically effective dose of tracer, the uptake data may be influenced by the tracer itself, even when working with very high specific activities.

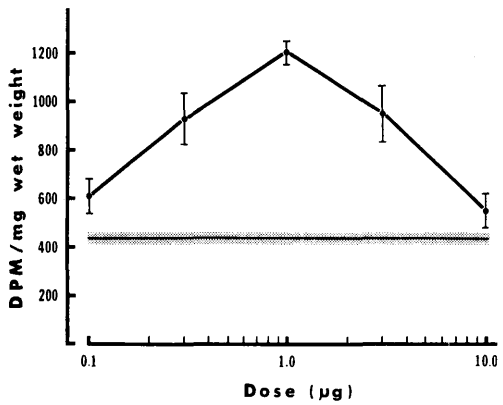

FIG. 4. A dose response of the priming effect of estradiol-17α. All groups received the various doses of estradiol-17α 6 hr prior to sacrifice and 1 μCi (6.4 ng) of 6, 7 ^{3}H-estradiol-17β 1 hr before sacrifice. The control value ± standard error is indicated by the shaded area. There were 10 animals in each group.

Jensen and co-workers (9) reported that mestranol must be demethylated by the liver in order to exert its effects on the uterus. Possibly this accounts for the greater dose requirement to produce its priming effect. However, there does not seem to be any difference in the timing of the response, as maximum is reached at about 3 hr and the effect is plateaued until 6 hr.

Priming appears to make more of a subsaturating dose of estradiol available to the uterus, possibly through its effect on transport and permeability. In 1938 Astwood (10) emphasized the estrogen-stimulated uptake of water by the uterus, which preceded true growth. Szego and Roberts (3) showed that estriol was more effective than estradiol in this respect. The similarities between the priming effect and water imbibition suggests a possible relationship between these estrogen responses.

The physiological significance of these findings is not yet established. The fact that estriol is a very weak uterotropic agent, and yet is able to exert such profound effects on early estrogen responses suggests at least two different sites of action with specific structural requirements. Miller and Emmens (11), studying ^{3}H-uridine incorporation into mouse uteri reported that estriol did not exhibit its impeded action on this early estrogen response. It was suggested that estriol and estradiol might be similarly effective at an initial site of action, while a second site

may be activated only by estradiol. The role of estriol as a physiological regulator of the action of estrogen was proposed by Hisaw (12). Since estriol inhibits estradiol binding, as demonstrated by Eisenfeld and Axelrod (5), high levels of estriol could reduce the accumulation of estradiol. Our results show that small quantities of estriol increase the uptake of estradiol by the uterus. Thus, estriol may have a dual role of enhancing available estrogen at low levels and of antagonizing secondary action of estradiol at high levels by competitive inhibition.

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Received June 30, 1969. P.S.E.B.M., 1970, Vol. 133.