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THE NATURALLY OCCURRING C-17 FATTY ACID ESTERS OF ESTRADIOL ARE LONG-ACTING ESTROGENS

JANICE M. LARNER, NEIL J. MACLUSKY and RICHARD B. HOCHBERG[1]
Yale University School of Medicine, Departments of Obstetrics and Gynecology and Molecular Biophysics and Biochemistry, 333 Cedar Street, New Haven, CT 06510, U.S.A.

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Summary—C-17 fatty acid esters of estradiol are naturally occurring biosynthetic metabolites of estradiol. A representative component of this family of esters, estradiol-17-stearate, was studied in order to determine the estrogenic properties of these unusual hydrophobic steroids. Following the classical estrogen bioassay, a solution of this ester in oil was injected subcutaneously into immature rats once a day for 3 days. There was little effect on the uterus on the first day after the third injection. However, on subsequent days a large stimulation of uterine growth occurred. The course of this estrogenic effect was exactly opposite to that obtained with estradiol. In order to eliminate the possibility that this effect on the time course of estrogenic stimulation was caused by increased solubility of the hydrophobic esters in the carrier oil, the steroids were administered to adult ovariectomized animals in aqueous medium via a single intravenous injection. The uterotrophic response to estradiol was maximal at 12 h and was completely dissipated in 48–60 h. Estradiol-17-stearate produced a uterotrophic effect of twice the duration of estradiol. In the immature rat, aqueous intravenous injections of estradiol-17-stearate produced a greater uterotrophic effect than estradiol and this effect was still maximal 96 h later. In addition, this single injection of estradiol-17-stearate advanced the time of vaginal opening, a marker for puberty in the female rat.

The mechanism of the prolonged estrogenic stimulation was investigated by studying the steroidal content of the uterus after injecting [^{3}H]estradiol and [^{3}H]estradiol-17-stearate i.v. into immature rats. At 1 and 4 h there was significantly more radioactivity in the uteri of the [^{3}H]estradiol treated animals. At later times (8 h and onwards) the total radioactivity in the uterus did not differ appreciably between the two groups. However at these later times, the amount of [^{3}H]estradiol was far greater in the uteri of animals receiving [^{3}H]estradiol-17-stearate. Consequently, the prolonged estrogenic effects of the endogenous C-17 fatty acid esters of estradiol are caused by the increased duration of the estrogenic signal. It is hypothesized that one of the roles of the fatty acid is to protect the steroid nucleus from metabolism and thereby prolong the life of the parent C_{18} steroid. Thus, the results of these experiments are consistent with this family of endogenous alkyl esters of estradiol having a physiological role as long-acting estrogens.

INTRODUCTION

For over fifty years synthetic alkyl and aryl esters of estrogens have been known to provoke prolonged estrogenic stimulation [1] and for this reason these pharmacologic estrogens are still used therapeutically [2]. Unexpectedly, estrogens which are similar to the synthetic esters have recently been shown to exist in nature. This laboratory found that when estradiol is incubated with various estrogen dependent tissues a non-polar compound is formed which is converted back into estradiol with hydrolytic treatment [3]. This hydrophobic metabolite which was called a lipoidal derivative of estradiol was subsequently identified as a family of C-17 fatty acid esters of estradiol [4]. More recently we have shown that these fatty acid esters of estradiol circulate in human blood [5].

The existence of these compounds posed the question of their physiological significance. While it is known that the synthetic C_{18} steroidal esters are active estrogens, it is generally believed that they produce their estrogenic effects only after hydrolysis *in vivo* to estradiol. In confirmation, we have recently reported that various C-17 alkyl esters of estradiol from C_2 to C_{20}, are not ligands for the estrogen receptor and therefore can not stimulate estrogenic responses directly through this mediator of estrogen action [6]. In addition, we found that while the short chain, synthetic C-17 esters were extensively hydrolyzed by incubation with uterine preparations, the fatty acid esters of estradiol were almost unaffected. The structural similarity of the naturally occurring esters to the synthetic pharmacologic esters, such as estradiol acetate, butyrate, etc. pointed to the possibility of an endogenous family of long-acting estrogens. However, the resistance of these fatty acid esters to enzymatic hydrolysis was an indication that they might not be "activated" by cleavage to estradiol *in vivo* and thus could not be potent estrogens. The experiments described in this paper investigate the estrogenic properties of a representative member of this family, estradiol-17-stearate.

[1]To whom correspondence should be addressed.

EXPERIMENTAL

Twenty-two day old female Sprague–Dawley rats, weighing 35–40 g, were purchased from Charles River Breeding Laboratories (Wilmington, MA). Animals were maintained under controlled temperature (22°C) and photoperiod (illumination, 0700–1900 h) and provided with food and water *ad libitum*. Estradiol was obtained from Steraloids (Wilton, NH) and stearoyl chloride from Nu Chek Prep (Elysian, MN). Estradiol-17-stearate was synthesized as described previously [4].

Two methods of hormone administration were employed. In the subcutaneous method, one-third of the total steroid dose was administered in the morning, daily, over 3 days in 0.1 ml of sesame oil. Control animals received sesame oil. In the intravenous method, the total dose of steroid was administered in 0.1 ml vehicle via the tail vein. Steroids were dissolved in ethanol prior to the addition of 4 vol of 0.9% NaCl containing 3% bovine serum albumin (Sigma, St Louis, MO). Control animals received only vehicle. At the appropriate times following hormone injections, animals were sacrificed by cervical dislocation. The uteri were removed, dissected free of extraneous tissue, carefully blotted and weighed. Uteri containing fluid were slit longitudinally and the accumulated fluid was removed prior to weighing.

Adult rats were ovariectomized at 45–50 days and injected intravenously 5 days later with 134 nmol/kilo body weight of estradiol or estradiol-17-stearate. Animals were killed and their uterine weights determined as described for the immature animals.

Uterine retention of [^3H]estradiol and [^3H]estradiol-17-stearate

Animals were injected intravenously with [6,7-^{3}H]estradiol or [6,7-^{3}H]estradiol-17-stearate (5.3 nmol/rat; sp. act. 4.4 Ci/mmol) and killed by decapitation at various time intervals between 1 and 48 h later. Uteri were removed, immediately chilled on ice, then cleaned and weighed. Individual uteri were homogenized in 1.5 ml of TE buffer and a nuclear-myofibrillar pellet prepared. The homogenates were centrifuged for 10 min at 800 *g*. The crude nuclear-myofibrillar pellet was washed 3 times with 2 ml of TE buffer (10 mM Tris, 1.5 mM disodium EDTA, adjusted to pH 7.4 with HCl) then resuspended in a further 2 ml of TE buffer and filtered through nylon gauze (0.2 mm mesh). The supernatant and the wash fractions obtained at each stage in the preparation of the nuclei were combined and collected on ice. These supernatants were termed the post-nuclear preparation. Following the addition of 100 μg estradiol as internal standard, one-third of each post-nuclear preparation was counted for radioactivity. The remainder were extracted 3 × with equal volumes of ether. The ether phases were combined and aliquots were evaporated and counted for radioactivity. The remainder were analysed by high pressure liquid chromatography (HPLC). The HPLC analyses employed a Waters unit equipped with a septum-less injector and a u.v. detector (280 nm). The ether was evaporated and the residues dissolved in 200 μl of CH_2Cl_2/isooctane (60:40) and injected onto a 10 μm LiChrosorb Diol column (Merck, 4.6 × 250 mm). The chromatogram was run at a flow rate of 1 ml/min with CH_2Cl_2/isooctane (60:40) and after 10 min the solvent was changed to CH_2Cl_2 for the remainder of the analysis. Fractions eluting with the internal standard were collected, dried and counted for radioactivity.

The nuclear-myofibrillar pellets were resuspended in 1.5 ml ethanol containing 100 μg estradiol and left overnight at 4°C. The alcoholic suspensions were centrifuged at 1700 *g* for 10 min and the ethanol decanted. A 0.5 ml aliquot of each extract was counted for ^{3}H and the remainder was evaporated and analyzed by HPLC as described above for the post-nuclear fraction. Essentially all of the radioactivity present in the nucleus was extracted into the ethanol. For these experiments the unconjugated fraction was defined as the ether and ethanol extractable radioactivity. If conjugated steroids were present in the nucleus they would have been extracted into ethanol, but almost all of the radioactivity in the nuclear fraction was unmetabolized estradiol.

The total radioactivity in the tissue was calculated by adding the ethanol extracted ^{3}H from the nuclear pellet to the radioactivity present in the post nuclear supernatant. Similarly, the total solvent extractable ^{3}H was determined by adding the ethanolic extract of the nuclear pellet to the ether extract of the post-nuclear fraction. Total [^{3}H]estradiol was calculated from the sum of the [^{3}H]estradiol found by HPLC in both fractions, after each was corrected for recovery of the internal standard.

Statistical analysis

Results were analysed using one-way analysis of variance and Duncan's multiple range test. Where necessary, the data were converted to their natural logarithms prior to analysis, to eliminate inhomogeneity of variance.

RESULTS

Uterotrophic effect of estradiol and estradiol-17-stearate administered subcutaneously in oil

The effects on uterine weight of increasing doses of estradiol and estradiol-17-sterate injected subcutaneously in oil are illustrated in Fig. 1. On the first day after the final injection (Day 1) the estradiol treated animals exhibited marked dose-dependent increases in uterine weight, compared to the control group. Thereafter the uterine weight of the estradiol treated animals declined rapidly. In contrast, the uterotrophic effect of the ester, estradiol-17-stearate, was minimal on day 1. The small effect at the higher doses was much less than that seen with estradiol. On days

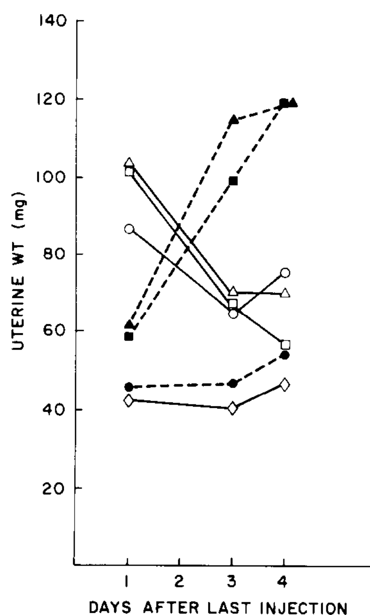

Fig. 1. The uterotrophic effect of estradiol and estradiol-17-stearate injected subcutaneously in sesame oil. Immature rats were injected on 3 consecutive days with a total of: ○, 5 nmol; □, 25 nmol; △, 50 nmol of estradiol or ●, 5 nmol; ■, 25 nmol; ▲, 50 nmol of estradiol-17-stearate, dissolved in 0.3 ml sesame oil. Controls, ◇, received 0.3 ml of sesame oil. Each point represents the mean of results from either 3 (1 and 4 days after the last injection) or 6 (3 days after last injection) animals. Statistical analysis: Analysis of variance revealed significant effects of the steroid treatments on days 1, 3 and 4 (day 1 $F = 26.7$; $df = 6, 14$; $P < 0.001$; day 3 $F = 7.28$; $df = 6, 35$; $P < 0.001$; day 4 $F = 10.17$; $df = 6, 14$; $P < 0.001$). Results for all three estradiol dose levels were significantly higher than vehicle controls on days 1 and 3 (day 1 $P < 0.01$; day 3 $P < 0.05$; Duncan's Multiple Range test). On day 4, only the 5 nmol estradiol group remained significantly above vehicle levels ($P < 0.05$). Uterine weights in the animals treated with the 25 and 50 nmol doses of estradiol-17-stearate were significantly higher than vehicle on all three days (day 1 $P < 0.05$; days 3 and 4 $P < 0.01$). There were no significant differences between the vehicle and 5 nmol estradiol-17-stearate treated animals on any day.

3 and 4, however, while the uteri of the estradiol treated animals were rapidly regressing, the animals treated with the 2 higher doses of estradiol-17-stearate were increasingly stimulated. The effects seen with the ester on days 3 and 4 were similar to that found with estradiol on day 1.

Uterotrophic effect of estradiol and estradiol-17-stearate administered intravenously in aqueous media

In order to control for effects that might be caused by prolonged retention of the lipophilic ester in sesame oil, further studies were performed in which the estrogens were administered intravenously in aqueous medium. The uterotrophic effect in immature rats of increasing doses of estradiol and estradiol-17-sterate, is shown in Fig. 2. Uterine weights were measured 24 h following injection. At that time the uterotrophic response to estradiol was

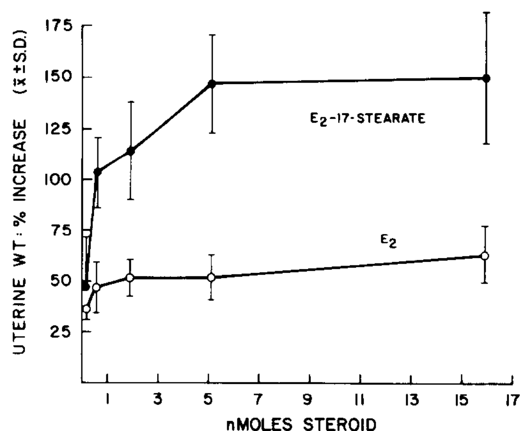

Fig. 2. The uterotrophic response of immature rats 24 h after a single intravenous injection of estradiol or estradiol-17-stearate in aqueous media. Values represent the mean ± SD of results obtained from five animals and are expressed as percent increase over vehicle injected animals. Mean uterine weight in the vehicle controls was 22.2 mg. (±2.6 mg; SD; $N = 10$). Uterine weights in all of the steroid-treated groups were significantly greater than in the vehicle controls ($P < 0.05$; Duncan's Multiple Range test).

small with a maximum between 1.8 and 5.3 nmol estradiol. The estradiol-17-stearate treated groups exhibited a sharp increase in uterine weights between 0.2 and 0.6 nmol, reaching a maximum at the 5.3 nmol dose (134 nmol/kilo b.wt).

The dose of 134 nmol/kilo of estradiol-17-stearate which produced a maximum uterotrophic effect at 24 h was chosen to study the time course of uterine stimulation. The response to the free steroid, estradiol, was rapid, peaking at 12 h, declining at 24 h and reaching control weights by 48 h (Fig. 3). The uterotrophic response to estradiol-17-stearate was

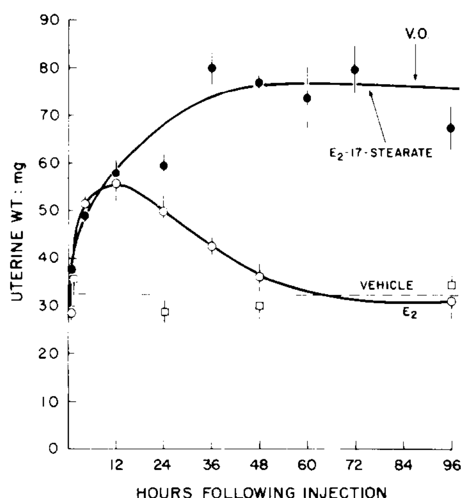

Fig. 3. Immature rats: the time course of the uterotrophic response to a single intravenous injection of 5.3 nmol of estradiol or estradiol-17-sterate. Steroids (5.3 nmol/rat) were administered in aqueous media as described in the text. Each point represents the mean ± SEM of results from at least 4 animals. V.O. indicates vaginal opening.

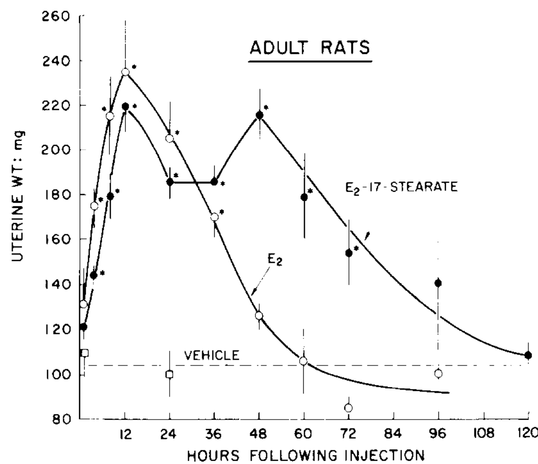

Fig. 4. Ovariectomized adult rats: The time course of the uterotrophic response to a single intravenous injection of 134 nmol/kilo b. wt estradiol or estradiol-17-stearate. Steroids were administered in aqueous media as described in the text. Each point represents the mean ± SEM of the following numbers of animals: Vehicle (□), 14; estradiol (○), 1 h = 5, 4 h = 6, 8 h = 6, 12 h = 10, 24 h = 13, 36 h = 6, 48 h = 10, 60-96 = 4 each; estradiol-17-stearate (●), 1 h = 14, 4 h = 20, 8 h = 14, 12 h = 13, 24 h = 18, 36 h = 14, 48 h = 26, 60 h = 12, 72 h = 8, 96 h = 4, 120 h = 4. *Significantly greater than vehicle controls (Duncan's Multiple range test, $P < 0.05$ level).

also measurable at 4 h, but in contrast to estradiol treatment, the uterine weights of the estradiol-17-stearate treated group continued to increase after 24 h, reaching a plateau at 36 h and remained maximally elevated even at the last time point measured, 96 h. Several groups of animals were also examined for vaginal opening, a marker for puberty in rats [7]. Six of eight estradiol-17-stearate animals had undergone vaginal opening by 96 h, day 26 of life. Contrariwise, 7 of 8 animals receiving estradiol had vaginal opening on day 28 and 7 of 8 vehicle controls on day 30.

In order to eliminate the effect of the estrogen ester on the stimulation of endogenous ovarian estradiol secretion, the uterotrophic effect of the same dose of the 2 estrogens was measured kinetically in adult ovariectomized rats. In this experiment the time course of the estradiol effect (Fig. 4) was similar to that found in the immature animals. While a greatly prolonged estrogenic effect was still obtained with estradiol-17-stearate, uterine weight declined to control levels by 120 h after injection.

Uterine retention of [^{3}H]steroids

The preceding experiments revealed a striking difference between estradiol and estradiol-17-stearate in temporal estrogenic stimulation. In order to investigate the mechanism by which the steroidal ester produces this prolonged effect a further experiment was performed in which [6,7-^{3}H]estradiol or [6,7-^{3}H]estradiol-17-stearate (5.3 nmol, 22 μCi) were injected into immature rats and the retention of the [^{3}H] steroids in the uterus and the uterine nuclei was studied. The results of the experiment measuring total uterine radioactivity are shown in Table 1. As would be expected for the free steroid, estradiol, the greatest amount of radioactivity found in the uterus was present at the earliest time point, 1 h. Of this, about $\frac{1}{2}$ (57%) was unconjugated since it could be extracted with organic solvents. Almost all of the solvent extractable radioactivity at 1 h was free estradiol. At 4 h the amount of radioactivity incorporated in the uterus had already fallen by approx 50% and of the total radioactivity only 21% was non-conjugated (extracted with organic solvents). For the next 8 h there was a plateau in the amount of radioactivity in all 3 fractions, total, non-conjugated and estradiol. Thereafter, there was a progressive decrease in the total amount of radioactivity in the uterus. In the interval between 36 and 48 h a plateau of low levels of non-conjugated steroid and estradiol was reached.

The results obtained with [^{3}H]estradiol-17-stearate were very different from that with the free steroid. At 1 h, the time at which maximal incorporation was found with [^{3}H]estradiol, there was only a relatively small amount of radioactivity in the uteri of animals

Table 1. Uterine radioactivity following the injection of [^{3}H]estradiol and [^{3}H]estradiol-17-stearate

Time (Hours)	Steroid injected	Total [^{3}H] dpm/uterus	Non-Conjugated [^{3}H]* dpm/uterus	[^{3}H]E_2 dpm/uterus
1	[^{3}H]E_2	19990 ± 4590	11340 ± 1530 (57%)	8710 ± 1460
	[^{3}H]E_2-17-Stearate	2850 ± 270	1510 ± 110 (53%)	790 ± 30
4	[^{3}H]E_2	10800 ± 960	2270 ± 280 (21%)	1700 ± 290
	[^{3}H]E_2-17-Stearate	6300 ± 70	5390 ± 730 (86%)	3000 ± 580
8	[^{3}H]E_2	10370 ± 270	1280 ± 100 (12%)	930 ± 30
	[^{3}H]E_2-17-Stearate	8060 ± 790	5660 ± 590 (67%)	4180 ± 500
12	[^{3}H]E_2	9500 ± 380	1290 ± 140 (14%)	860 ± 130
	[^{3}H]E_2-17-Stearate	10380 ± 180	5040 ± 530 (49%)	4620 ± 380
24	[^{3}H]E_2	4680 ± 430	890 ± 160 (19%)	600 ± 60
	[^{3}H]E_2-17-Stearate	5910 ± 20	2450 ± 30 (48%)	2600 ± 210
36	[^{3}H]E_2	2080 ± 110	460 ± 60 (22%)	160 ± 30
	[^{3}H]E_2-17-Stearate	2290 ± 190	1550 ± 150 (68%)	900 ± 110
48	[^{3}H]E_2	1160 ± 140	530 ± 40 (46%)	150 ± 30
	[^{3}H]E_2-17-Stearate	2030 ± 190	1590 ± 130 (78%)	960 ± 50

Immature rats were injected i.v. with (5.3 nmol, 22 μCi) of [^{3}H]estradiol or [^{3}H]estradiol stearate. The solvent extractable radioactivity as well as the estradiol were calculated from the sum of the nuclear and post-nuclear fractions. The values in parentheses are the percentage of total radioactivity that is solvent extractable. Values are the mean ± SD. $n = 3$. *Non-conjugated ^{3}H is defined as the solvent extractable radioactivity. For details see Experimental section.

treated with [^{3}H]estradiol-17-stearate, approx 15% of that obtained with free steroid. Contrary to the results with estradiol, the amount of radioactivity in the uterus of the estradiol ester treated animals increased up to 12 h, then gradually declined. Strikingly, at every time point, the amount of unconjugated radioactivity was at least 50% of the total present in the uterus. The greatest difference between the free and the esterified steroid was seen in the unconjugated fraction and in [^{3}H]estradiol. For example, at 4 h, while there was almost twice as much total uterine radioactivity in the estradiol treated animals as in the rats treated with estradiol-17-stearate, there was twice as much non-conjugated radioactivity and estradiol in the estradiol-17-stearate treated animals. At 12 h, the time at which there was approximately the same amount of total radioactivity present in the uteri from both groups, the difference in the non-conjugated fraction became even greater. At this time there was four times as much non-conjugated radioactivity and 6 times as much [^{3}H]estradiol in the estradiol stearate treated animals. This difference was maintained even at the last time point studied (48 h), at which time there was but 150 dpm of [^{3}H] estradiol per uterus in the estradiol treated animals compared to 960 dpm of [^{3}H]estradiol in the animals administered estradiol-17-stearate.

Essentially all of the radioactivity in the nuclear pellets was [^{3}H]estradiol. The data in Fig. 5 show that in the animals injected with [^{3}H]estradiol, the amount of [^{3}H]estradiol found in the nucleus was already maximal at 1 h and thereafter it decreased precipitously, until at 12 h only about 15% of the radioactivity present at 1 h was still in the nucleus (Fig. 5). Contrariwise, the amount of [^{3}H]estradiol found in the nucleus of the rats treated with [^{3}H]estradiol-17-stearate was not maximal until 8–12 h after the injection. At 24 h, the amount of nuclear [^{3}H]estradiol was still more than 50% of that seen at 8–12 h. At 24 h there was seven times more [^{3}H]estradiol in the nucleus of the estradiol ester treated animals as in those treated with the free steroid. At the last time point, 48 h, there was still a 5-fold difference.

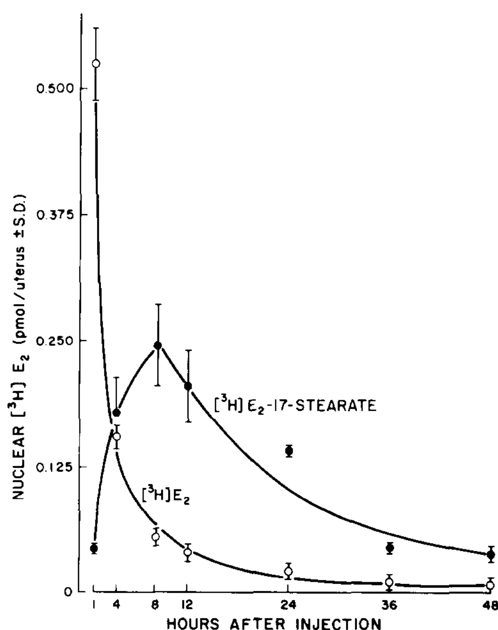

Fig. 5. [^{3}H]Estradiol content of rat uterine nuclei following a single intravenous injection of 22 μCi (5.3 nmol) [^{3}H]estradiol or [^{3}H]estradiol-17-stearate. Each point represents the mean ± SD of results obtained from three animals.

DISCUSSION

The present experiments show that estradiol-17-stearate is an estrogenic steroid with a prolonged action. When injected subcutaneously in oil the uterotrophic effect of the ester was still maximal 3 and 4 days later, at which time uteri of estradiol treated animals had regressed dramatically (Fig. 1). When injected in oil the ester is barely uterotrophic on day 1 which is the time of maximal stimulation of the free C_{18} steroid. It has been suggested that the prolonged estrogenic action of synthetic estradiol esters, like estradiol benzoate, is caused by their decreased rate of resorption from oil depots [8]. This might have been the cause of the delay in the uterotrophic stimulation seen with estradiol-17-stearate (Fig. 1) but it would not explain its prolonged effect because this effect was still apparent when the steroidal ester was administered in aqueous media (Figs 3, 4). With this latter paradigm the uteri of the animals receiving estradiol were maximally stimulated at 12 h and had regressed to baseline at about 48 h. Contrariwise, the uteri of the immature animals receiving estradiol-17-stearate were not maximally stimulated until 36 h after the administration of the steroid and the effect was maintained for at least 96 h, the last point measured. In adult animals the initial uterine response with the ester was similar to that with estradiol (Fig. 4). However, in contrast to the uteri of the estradiol treated animals, which regressed rapidly after 12 h, the uterine weight of the adult animals treated with the ester remained stable between 24 and 36 h, increased significantly at 48 h ($P < 0.05$. 48 h vs 24 h and 36 h; Duncan's multiple range test), decreasing to control levels only at 120 h.

The prolonged and constant estrogenic stimulation of the estradiol-17-stearate treated immature animals is also evident from the fact that they reached puberty prematurely, as evidenced by vaginal opening. Vaginal opening occurred at 26 days of life in these animals (Fig. 3), 2 days before those receiving estradiol and 4 days before the controls. This is probably the reason why the uteri of the estradiol-17-stearate treated animals do not return to the unstimulated state. Part of the prolonged uterotrophic effect of the estradiol ester on immature animals can probably be ascribed to pituitary stimulation inducing the immature animals' ovaries to produce

estrogen. It is well known that repeated administration of estradiol to immature rats will stimulate the hypothalamic-pituitary axis to secrete gonadotrophins, thereby accelerating vaginal opening [7, 9]. The response to a single injection of the ester seems to mimic the effect of multiple injections of estradiol. The uterotrophic effect of the ester in mature ovariectomized animals is consistent with this hypothesis (Fig. 4). Unlike the intact immature rat, the ovariectomized adult uterine weight does eventually return to base line after the injection of estradiol-17-stearate. The uterine weight in the mature ovariectomized animal remains significantly elevated for 72 h after the injection of estradiol-17-stearate, twice as long as estradiol. The biphasic nature of the response to estradiol-17-stearate seen at 48 h probably reflects sensitization of the uterus which results in a later enhanced effect of the continued estrogenic stimulus [10, 11].

The subsequent experiment with ^{3}H-labelled steroids clearly shows that there is a difference between estradiol and estradiol-17-stearate in the long-term uterine nuclear retention of estradiol. In this experiment, radioactive estradiol and estradiol-17-stearate were injected into immature rats and the nature of the radioactivity in the uterus and the uterine cell nuclei was determined. A delayed uptake of ^{3}H in the uterus as well as the nucleus was seen in those animals treated with [^{3}H]estradiol-17-stearate, compared to those injected with [^{3}H]estradiol (Table 1, Fig. 5). In addition, there was a prolonged retention of radioactivity in the uterus of the [^{3}H]estradiol-17-stearate treated group. This increased radioactivity was especially pronounced when comparing total unconjugated steroids (organic solvent extractable) or estradiol. In every case, at times greater than 4 h after the injection, there was approximately the same amount of total ^{3}H in the uteri of the 2 groups but there were always large differences in the amounts of total unconjugated steroid and estradiol. When the [^{3}H]estradiol present in the nucleus was measured (Fig. 5), the delay at 1 h and the increased retention of the C-17 ester treated animals were obvious. Even at 48 h, the last time point measured, there was as much or more [^{3}H]estradiol present in the uterine nuclei of the [^{3}H]estradiol-17-stearate treated animals as in the [^{3}H]estradiol treated animals at 12 h.

It is clear that not only is there a lag in estrogen uptake (Table 1) with estradiol-17-stearate but, more importantly, there is also prolonged retention of the estrogenic signal in the target organ (Table 1) and nucleus (Fig. 5) with this steroidal ester. The delay in estradiol uptake with the ester could be due to any of a multitude of causes, including transport of estradiol.17.stearate into the cell. Since C-17 fatty acid esters of estradiol do not bind to the receptor [6] and because virtually all of the radioactivity in the uterine nuclei in animals injected with [^{3}H]estradiol-17-stearate is [^{3}H]estradiol, it appears likely that all of the estrogenic effects of estradiol-17-stearate follow the enzymatic hydrolysis of the ester to estradiol. Thus the lag seen in Table 1 and Fig. 5 most likely reflects a slow release of the free steroid from the ester.

In all, it is apparent that this family of fatty acid esters of estradiol, of which estradiol-17-stearate is only one component, has some unique properties associated with prolonged action. The data in Table 1 show a relatively large proportion of [^{3}H]estradiol when compared to total radioactivity, in the uteri of the [^{3}H]estradiol-17-stearate treated animals. This large amount of intact, free C_{18}-steroid suggests that a function of the fatty acid in the ester is to protect the steroid nucleus from metabolism. If a low metabolic clearance from blood and slow hydrolysis in tissues is concurrent with the protective effect, then this combination would be a likely mechanism for the sustained estrogenic action of these esters.

The discovery that fatty acid esters of estradiol are naturally occurring compounds [3–5] provided the impetus for the present studies, which show that these esters have the unusual property of providing a sustained estrogenic signal. The physiological significance of these findings remains to be established, but a probable hypothesis is that these compounds may serve to provide some estrogenic stimulation under conditions were ovarian output of estradiol has either temporarily ceased or is fluctuating. Another important consideration is that pathological expression of the estradiol esters is possible and many contribute to abnormal stimulation of estrogen target tissues.

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