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Plasma Concentrations of Estradiol Produced with Two Delivery Systems in Ovariectomized Rats[1] (40229)

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In research involving treatments with estradiol-17β (E_2), daily injections of E_2 in oil are often used under the supposition that slow absorption and prolonged circulation of the hormone occurs. Estimates of physiological dosage which have been based on biological responses of target organs, vary depending on end point (1), age of the animal (2) and length of photoperiod (3). Using daily injections of E_2 in 0.1 ml sesame oil, Parlow (1) found the apparent daily physiological dosage of estradiol for ovariectomized immature rats to vary from 0.1 μg with uterine weight as the end point to >2.0 μg for suppression of pituitary concentration of FSH. Daily dosages of E_2 from ng to mg levels are reported in literature for rats without apparent knowledge of circulating concentrations or patterns of E_2 produced. Goodman (4) has reported constant serum concentrations of E_2 for 6 days following implantation into ovariectomized rats of Silastic capsules containing E_2, but plasma levels after injections of E_2 in oil have not been reported for rats. Rapid elevation of E_2 in plasma with a short duration was found in ewes and cows following an im injection of E_2 in corn oil (5).

In the present study, the concentrations and patterns of E_2 in plasma were investigated following a single injection of E_2 in sesame oil or during a constant release of E_2 from osmotic minipumps.

Materials and methods. Sprague-Dawley rats weighing 215–225 g and exhibiting 4- or 5-day estrous cycles were ovariectomized. Seven days after surgery, 24 rats were assigned to four groups of six animals. Rats received a single sc injection of either 0, 0.25, 0.5 or 1.0 μg estradiol-17β (E_2) in 0.1 ml of sesame oil. One ml of blood was collected by cardiac puncture under ether anesthesia from each rat at 2, 4, 8 and 12 hr after treatment.

Two additional groups of 5 rats each received either 1 or 2 Alzet (R) osmotic minipumps (Alza Corporation, Palo Alto, CA) subcutaneously in the back of the neck. These minipumps were filled with 170 μl of propylene glycol containing 45.788 μg E_2/ml and had a delivery rate of 0.91 μl/hr. This gave a delivery rate of 1 μg E_2/24 hr. Minipumps were implanted in a host rat overnight to establish a constant flow rate before being placed in the experimental animals. One ml of blood was collected by cardiac puncture under ether anesthesia at 3, 6, 12, and 24 hr after insertion of the minipumps.

Cardiac puncture was accomplished by a 22 gauge needle and 1 ml syringe with the blood aspirated into a 10 × 75 mm culture tube containing 25 units of heparin. Following centrifugation at 1000g for 30 min at 4°, the plasma was stored frozen until assayed for E_2. Description and verification of the radioimmunoassay and chromatography for E_2 have been reported (6, 7). The concentrations of E_2 were determined from 0.4 ml of plasma. The sensitivity of the assay (1 pg) gave detection limits of 2.5–500 pg/ml.

Results. For all dosages at the times studied, E_2 was at maximal concentration in plasma at 2 hr after injections, declined about 50% by 4 hr, and had returned to control levels by 12 hr (Table I). At 2 hr after the 0.25 μg dose, the plasma concentration (66 ± 8 pg/ml) was as great as during the peak levels at proestrus (6). Dosages of 0.5 or 1.0 μg of E_2 produced plasma concentrations (104 ± 12 and 235 ± 18 pg/ml, respectively) at 2 hr that were far in excess of any levels occurring during the estrous cycle of the rat, yet concentrations had returned to control levels by 12 hr.

One minipump, delivering 1 μg E_2/24 hr, produced relatively constant levels (mean = 38 ± 3 pg E_2/ml) over the 24-hr collection

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period (Table II). Two pumps, delivering 2 μg E_2/24 hr, gave about twice the concentrations in plasma (mean = 84 ± 6 pg E_2/ml) of one pump.

Discussion. This study demonstrates that injections of E_2 in sesame oil at doses (0.5–1.0 μg) considered to be in the physiological range, produce peak levels of E_2 in the plasma of ovariectomized rats far in excess of peak concentrations found during estrous cycles; and circulating levels of E_2 return to basal levels in less than 12 hr. Therefore, use of daily treatment with E_2 in oil cannot provide physiological levels and at the same time maintain elevated plasma concentration of E_2. Estradiol benzoate (E_2B) often has been used to provide longer estrogenic action presumably by slower release from the injection site. Goodman (8) recently reported that >60% of E_2B is converted to E_2 in blood from rats within 30 min, which suggests that daily injections of E_2B would produce surge-type patterns of E_2 of short duration if absorption was rapid. Henderson *et al.* (9) found that injections at 2.5 or 10 μg of E_2B in oil produced peak concentrations of about 30 and 120 pg/ml, respectively, of E_2 in plasma at 18 hr and was followed by a rapid decline of E_2 levels. Rexroad (5) reported only slight, but prolonged, elevations in levels of E_2 in plasma of cows after injections of E_2B in oil. Together, these two reports indicate E_2B is absorbed more slowly from oil than E_2, and both are eliminated rapidly from circulation. The replacement dosage of E_2B for the adult rat (9) lies between 2.5 and 10 μg and provides more constant plasma levels of E_2 than E_2 injections, but less constant than Silastic implants or minipumps.

The minipumps provided a stable level of E_2 in plasma and, based on the two doses used in this study, produce plasma levels proportional to dosage delivered. These pumps can deliver this constant rate of 0.91 μl/hr for 7 days. Goodman (4) reported a constant level of E_2 for at least 6 days by implants of Silastic capsules containing E_2 in peanut oil. Silastic implants, minipumps or other constant release mechanisms would be a more desirable system for maintaining constant plasma levels of steroids than oil vehicles.

TABLE I. PLASMA CONCENTRATIONS OF ESTRADIOL-17β(E_2) FOLLOWING A SINGLE SC INJECTION IN SESAME OIL.

	Plasma E_2 concentration (pg/ml)			
Dosage E_2	+2 hr	+4 hr	+8 hr	+12 hr
Sesame oil	5 ± 2[b]	5 ± 1	5 ± 1	6 ± 1
0.25 μg	66 ± 8	28 ± 4	12 ± 1	8 ± 4[a]
0.5 μg	104 ± 12	56 ± 6	19 ± 5	6 ± 1[a]
1.0 μg	235 ± 18[a]	134 ± 13	26 ± 4	9 ± 1

[a] n = 5, all other n = 6.
[b] Mean ± SEM.

TABLE II. PLASMA CONCENTRATION OF ESTRADIOL-17β(E_2) FOLLOWING SC INSERTION OF OSMOTIC MINIPUMPS.

	Plasma E_2 concentration (pg/ml)			
Delivery rate E_2	+3 hr	+6 hr	+12 hr	+24 hr
1 μg/24 hr	29 ± 5[b]	38 ± 4	46 ± 9[a]	39 ± 3
2 μg/24 hr	90 ± 9	83 ± 10	79 ± 3[a]	81 ± 23

[a] n = 4, all other n = 5.
[b] Mean ± SEM.

Since chronic levels of E_2 suppress gonadotropins and an acute rise of E_2 releases LH, it is important that a delivery system provide the proper level and pattern of estrogen for a particular study. Unless the peak concentration and pattern of plasma estrogen resulting from treatment with this hormone is known, it is difficult to interpret results of such treatments. Therefore, before applying a hormonal treatment in which the pattern and/or concentration of the circulating hormone is important, the delivery system should be tested for both level and pattern of hormone produced in circulation.

Summary. Ovariectomized rats received a single sc injection of 0, 0.25, 0.5 or 1.0 μg of estradiol-17β (E_2) in 0.1 ml sesame oil or sc implants of osmotic minipumps delivering 1 or 2 μg E_2/24 hr. Plasma levels of E_2 were determined at 2, 4, 8 and 12 hr after injections or 3, 6, 12 and 24 hr after insertion of minipumps. Injections produced superphysiological levels of E_2 in plasma by 2 hr, but E_2 was depleted from plasma in less than 12 hr. Minipumps provided constant circulating concentrations of E_2. Constant release methods provide a much more logical delivery system for estrogen suppression of gonadotropin than the use of an oil vehicle.

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