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Accumulation of ^{3}H-Estradiol in the Vaginas and PituitaryGlands of Mice of Inbred Strains[1] (38658)

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The estrous cycles of mice of different inbred strains differ in the intervals between periods of vaginal cornification and the duration of vaginal cornification (1–5). Furthermore ovariectomized mice of different inbred strains show differences in the responsiveness of the vaginal mucosa to the injection of estrogens (6–10). Ovariectomized mice of the Strong A strain require approximately five times more estradiol benzoate to induce vaginal cornification than did mice of the C57 strain (10, 11). The strain differences in the sensitivity of the vaginal mucosa were attributed to intrinsic differences because when the vaginas of A and C57 strain donors were transplanted into their hybrid hosts, they retained their characteristic differences in responsiveness (11–13). When steroidal estrogens were applied topically to the vaginal mucosa much smaller amounts induced cornification than when injected subcutaneously (14). The same order of strain differences occurred after the topical application, an observation that showed that the strain differences were not due to differences in the capacity to inactivate or excrete the estrogen, but were intrinsic to the vagina (8–14).

Mice of the C57 strain have pituitary tumors following the injection of estrogens; estradiol and its esters, diethylstilbestrol, and triphenylethylene (15, 16). The pituitary levels of ^{3}H-E2 of males of the A and C57 strains at 1 hr after injection were not different (17). The concentration of ^{3}H-E2 in the vaginas and pituitary glands and other tissues at 1, 6 and 12 hr after injection is reported here.

Materials and Methods. Young adult mice of the A and C57 strains were ovariectomized when about 2 mo of age and 1–7 mo later were used to determine ^{3}H-E2 accumulation. Groups of 6–11 mice were included in each of three different experiments. They were removed at either 1, 6, or 12 hr after injecting ^{3}H-E2 subcutaneously in aqueous solution. The injections were timed so that the mice could be removed between 8 and 12 AM at 10-min intervals. The mice were caged in stainless steel cages, 6 × 6 × 12 in. and housed in a room at 78–80° F and 50% relative humidity and lights on and off 12 hr, 7 to 7 o'clock. Tap water and a 50/50 mix of Purina Mouse Chow and D and G Cooked Mouse Biscuits were available *ad libitum*. The mice in one experimental group had received repeated subcutaneous injections at intervals of 1–4 wk of 0.0125 to 0.1 μg estradiol-17β dissolved 0.05 ml/sesame oil to determine their vaginal responses. Vaginal smears were obtained beginning 48 hr after each injection and were continued until all smears indicated diestrus. Smears containing 90%, or more, completely cornified cells were classified as estrus and considered positive.

Chromatographically pure 6, 7 ^{3}H-E2 (38 Ci/mM) was obtained from New England Nuclear dissolved in methanol–benzene. An ethanol–water solution was added to the methanol–benzene solution and after flash evaporation the ^{3}H-E2 in aqueous solution was adjusted to the desired volume. Injections of the aqueous solution were made subcutaneously at 0.1 μg/100 g body wt. Three separate experiments were undertaken using groups of from 36 to 47 mice. Mice were killed by exanguination 1, 6, 12 or 24 hr after the injection of the ^{3}H-E2.

Prior to necropsy the mice were anesthetized with ether. Blood was obtained by sectioning the right carotid sheath and collected

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in small heparin-containing test tubes. The pituitary, vagina, uterus, heart, and in two experiments, the hypothalamus and a frontal portion of the cerebrum were removed, weighed, and placed in 2 ml H_2O in ice chilled screw-topped 15 ml tubes. Each tissue from each mouse was then homogenized separately and extracted with 10 ml toluene–isoamyl alcohol (19/1) to obtain the unconjugated estrogens. Five ml of the toluene–isoamyl alcohol extract was added to 10 ml PPO POPOP (2,5-diphenyloxazole and *p*-bis [2-(5-phenyloxazolyl)]-benzene) in toluene and counted in a Packard scintillation spectrophotomoter, Model 3320, 40% efficiency. The counts were corrected for the aliquot and an extraction efficiency of 85% and expressed in counts per minute per mg wet wt of the tissue (cpm/mg). This extraction and counting procedure has been described previously in more detail (18, 19). Student's *t* test was used for statistical evaluation.

The toluene–isoamyl alcohol extracts remaining after the 5 ml portion of each had been counted individually were pooled by experimental group and tissue and the unconjugated estrogens were separated chromatographically to determine the relative amounts of estrone, estradiol and estriol. Cold carrier estrone, estradiol and estriol were added to the pooled samples. After flash evaporation they were redissolved in methanol and evaporated to a small volume under nitrogen. The methanol solution was applied to Whatman No. 1 chromatographic paper and separation was done using the Bush 3 system (20, 21).

Observations and Discussion. The vaginal epithelium of 50% ± 10% of the mice of the C57 strain was cornified after the injection of between 0.0125 and 0.025 μg estradiol subcutaneously in a single dose. Mice of the A strain required approximately 0.1 μg estradiol for a comparable response. The strain difference was approximately five-fold and comparable to that observed after the injection of estradiol benzoate (10, 11).

The vaginas of mice of the more responsive C57 strain showed significantly more accumulation of ^{3}H-E2 in two of the three experiments at 1 hr after the injection of the tritiated estradiol (Fig. 1). Although the ^{3}H-E2 uptake was also greater in the other experiment the difference was not statistically significant. The retention ^{3}H-E2 over 6- or 12-hr periods was not significantly different in the two strains. The slight strain differences in the accumulation and retention of E2 do not seem sufficient to account for the differences in responsiveness.

The pituitary glands of mice of the C57 strain retained larger amounts of ^{3}H radioactivity for 6 and 12 hr than did the mice of the A strain. Only in experiment 1 were significant differences noted at 1 hr (Fig. 2). Mice of the C57 strain have tumors of the pituitary glands following prolonged ex-

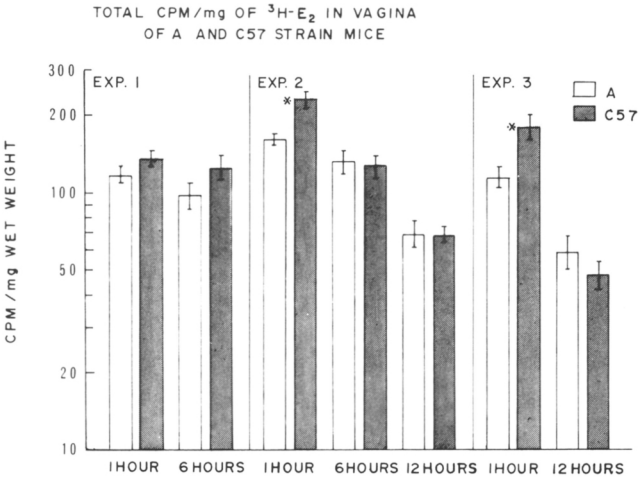

FIG. 1 The accumulation and retention of ^{3}H-E2 by the vaginas of mice of the A and C57 strains. Means and standard errors are shown. *P = 0.05.

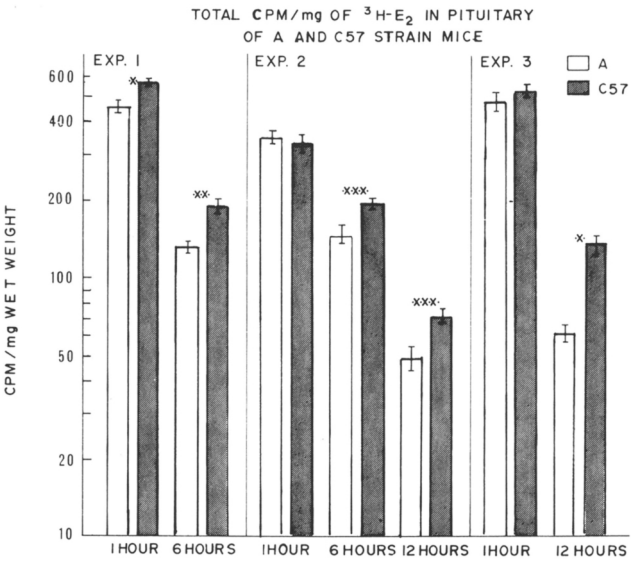

FIG. 2 The accumulation and retention of ^{3}H-E2 by the pituitary glands of mice of the A and C57 strains Means and standard errors are shown, $*P = 0.001$; $**P = 0.01$; $***P = 0.025$.

posure to estrogens (15, 16). Pituitary tumors have not appeared in estrogen treated mice of the A strain. It is conceivable that the higher concentration retained in the tumor susceptible C57 pituitary gland may be one contributory factor involved in the tumorigenesis. The current concepts of the early steps of estrogen action have been recently reviewed (24). The difference in pituitary estradiol kinetics also suggest the possibility that the molecular basis of estradiol interaction with a cytoplasmic receptor or its translocation and binding in the nucleus may not be identical in the two strains. The pituitary of the rat is known to contain cytosol estradiol binding proteins (23) and to have an ultimate nuclear localization of most of the estradiol (25). We have found that the anterior pituitary of the mouse also has ultimate nuclear binding as shown by autoradiography.

To determine whether the relative amounts of estriol, estradiol, or estrone might differ in the organs, pooled portions of the extracts from each organ of the different experimental groups of experiments 1 and 2 were chromatographed and the relative amounts of ^{3}H-E2 was slightly greater in the vaginas of the C57 mice but the number of observations is limited. The pituitary glands and the uteri showed no consistent differences in the amounts of unconjugated estrogen. There were no significant strain differences in the concentrations of ^{3}H-E2 in the hypothalamus, cerebrum, heart or plasma at 1 and 6 hr or of the uterus at 1, 6 and 12 hr after injection.

Summary. The sensitivity of the vaginal epithelium of ovariectomized mice of the A and C57 strains to estradiol differ approximately fivefold. The vaginas of the more sensitive C57 strain did accumulate more ^{3}H-E2 at 1 hr in two of three experiments but the retention at 6 and 12 hr was not significantly different. The pituitary accumulation of ^{3}H-E2 by the pituitary-tumor susceptable C57 mice, although not significantly greater than that of the nonsusceptable A strain mice at 1 hr was higher at 6 and 12 hr after the injection. Most of the toluene extractable radioactivity from the pituitary glands and vaginas was estradiol with no strain differences. The accumulation of ^{3}H-E2 in other tissues was not significantly different in mice of the two strains.

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