Critical Interaction Time Between Estrogen and Uterine Tissue Indicated by Cortisol Inhibition and Inhibition Release.* (27877)

EUGENE SPAZIANI (Introduced by J. J. Kollros)
Department of Zoology, University of Iowa, Iowa City

A recent report from this laboratory(1,2) examined cause-effect relationships between successive events in rat uterus resulting from short-term estrogen treatment. The adrenocortical hormone, cortisol, was utilized to inhibit the early vascular responses to estradiol (3,4) or to histamine with the objective of discerning the effects on uterine growth of diminishing or suppressing the vascular reactions. Cortisol administered at the same time as estradiol inhibited both the edema production (4 hours) and the cell division (24 hours) normally induced by estradiol alone. However, if cortisol injection was delayed until 6 hours after estradiol, the adrenal steroid no longer inhibited the growth response to the estrogen(2). The present report summarizes an extended study of the latter finding. Cortisol was given at varying time intervals following a minimal dose of estradiol to determine the point in time during which estrogen escapes cortisol inhibition. It was hoped information might be obtained concerning a critical time of interaction of estrogen with cells of a typical target organ.

Materials and methods. Adult rats (Sprague-Dawley) were bilaterally ovariectomized and maintained under controlled conditions of temperature and lighting (14 hours of artificial light and 10 hours of total darkness per day). Experiments were begun 14 days following operation between 2-3 P.M. (hour 0) on each day of experimentation. Estradiol-17β (0.1 μg per 100 g body weight) in saline solution (1 μg/ml) was given at the beginning of an experiment. Cortisol acetate in aqueous suspension(2) was administered in the amount of 3.5 mg per 100 g body weight (10 mg/ml) just prior to the estrogen injection or 1, 2, 3, or 4 hours after the estrogen. Control animals received either estradiol or cortisol and an injection of the carrier solution of the missing hormone at hour 0; a third control group was given a double injection of carrier and no hormones. All injections were given intravenously while the animals were under light ether anesthesia.

Animals were sacrificed by decapitation 6 hours or 24 hours after initial injection. In the 6-hour group, uterine horns were removed, stripped of extraneous tissue and gently blotted; water content was determined on these from the wet-dry difference and expressed as per cent of fresh tissue weight. Growth was measured in uteri from rats sacrificed 24 hours after initial injection. These animals received colchicine (Inland Alkaloid, powdered, USP) in the amount of 0.1 mg per 100 g body weight, subcutaneously, 6 hours before sacrifice. A segment of each uterine horn was fixed in Bouin's fluid, sectioned in paraffin at 8 μ and stained in haematoxylin and eosin. Growth was quantified by counting the total number of luminal epithelial cells arrested in mitosis, as well as the total number of luminal epithelial cells present, in one focal plane of each cross-section. Counts were made on 8-16 cross-sections per rat. Results were expressed as a mitotic index, *i.e.*, number of cells in mitosis/1000 cells. Four to six animals comprised an experimental group and Student's "t" test was used for statistical analysis of the data.

Results and discussion. In Fig. 1 are summarized the results of separate experiments in which cortisol was administered at increasing time intervals after a single dose of estradiol. Uterine water content or cell division was measured in animals sacrificed 6 hours or 24 hours, respectively, after initial injection. Cortisol exerted its greatest inhibitory influence on estradiol-induced water uptake or growth when administered 1 hour after estradiol. At this time interval

* Supported in part by research grant from U. S. Public Health Service.

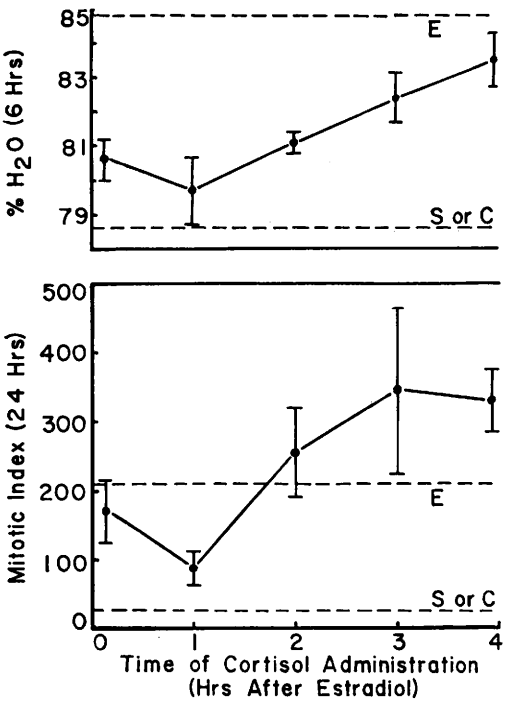

FIG. 1. Influence of time of cortisol administration on estrogen-induced uterine hydration and cell division. Water content and cell division determined in rats sacrificed 6 hr (upper graph) and 24 hr (lower graph), respectively, after estradiol injection (hour 0). Points are avg for uteri from 4-6 rats ± standard deviation. Dashed lines in each graph represent results for controls given estradiol (E), cortisol (C) or saline (S) alone at hour 0.

(Fig. 1), both water uptake and cell division were depressed significantly below the values for estradiol alone ($P<0.001$ and <0.05, respectively). The value at 1 hour for water uptake, moreover, cannot be distinguished statistically from the control level of saline or cortisol alone ($P<0.2$).

In contrast, cortisol administered 2 hours after the estrogen did not inhibit growth (Fig. 1). Water uptake at the 2-hour interval, though still depressed below the level for estradiol alone, was no longer completely inhibited by cortisol; water content was elevated significantly above the saline-cortisol control level ($P<0.01$). Delay of cortisol administration for 2 hours or for periods up to 4 hours after estradiol appeared to *enhance* the effect of the estrogen on growth, though the only statistically reliable results in this regard occurred at the 4-hour-delay interval ($P<0.05$). A similar tendency of cortisol to enhance the growth effect of estradiol was observed in a preliminary experiment in which cortisol was given intravenously 6 hours following injection of estradiol directly into the uterine lumen(2).

The earliest indications of uterine stimulation by estrogen in immature or ovariectomized adult rats appear within the first 2 hours following a single injection of the hormone. Thus, initial indications of small vessel dilation, edema, electrolyte and nonelectrolyte exchange and certain metabolic changes have been reported(3-6). The present findings are consistent with the expectation, therefore, that an estrogen antagonist such as cortisol would most seriously affect estrogen-induced uterine growth if applied within this early period following estrogen administration (*cf.* 2). The results suggest that the first 2 hours of estrogen interaction with uterine cells are critical especially in relation to the accelerated growth which follows. Introduction of an antagonist beyond the first 2 hours does not depress growth, suggesting further that the estrogen-sensitive sequence leading to acute growth effects, whatever that sequence may be *in vivo*, is self sustaining once set into motion and may not further require the presence of estrogen.

Summary. The effects of time of administration of cortisol, an estrogen antagonist, on estrogen-induced edema production and cell division were studied in uteri of ovariectomized rats. The adrenocortical hormone depressed or prevented these responses when given within 2 hours following estradiol administration; when injection was delayed longer than 2 hours, cortisol no longer completely inhibited the uterine hydration response to estradiol nor did it depress cell division. The results indicate that the acute (24 hour) growth effect, stimulated by estrogen in uterine epithelium, is dependent upon hormone-tissue interactions occurring within the first 2 hours following estrogen injection. Beyond this period, the stimulated sequence culminating in growth appears to be self sustaining and not further subject to cortisol antagonism.

1. Spaziani, E., *Fed. Proc.*, 1961, v20, 198.
2. ———, *Endocrinology*, 1963, in press.
3. Szego, C. M., Roberts, S., *Recent Progr. Hormone Res.*, 1953, v8, 419.
4. Spaziani, E., Szego, C. M., *Am. J. Physiol.*, 1959, v197, 355.
5. Mueller, G. C., Herranen, A. M., Jervell, K. F., *Recent Progr. Hormone Res.*, 1958, v14, 95.
6. Noall, M. W., Allen, W. M., *J. Biol. Chem.*, 1961, v236, 2987.

Received September 26, 1962. P.S.E.B.M., 1962, v111.

Nucleic Acid Estimates of Mammary Tissue and Nuclei.*† (27878)

H. ALLEN TUCKER AND RALPH P. REECE
Department of Dairy Science, New Jersey Agricultural Experiment Station, New Brunswick

The use of deoxyribonucleic acid (DNA) content as a measure of cell numbers was first suggested by Davidson and Leslie(1). DNA and ribonucleic acid (RNA) content of mammary tissue have been used, respectively(2,3), as indices of growth and of metabolic activity. DNA as an index of growth is valid only when DNA content per nucleus remains constant(1). DNA per nucleus was found to be constant in mammary and pooled kidney and spleen tissue during pregnancy but at parturition it declined sharply and it was not until late lactation that it approached normal(4). Folley(5) recently expressed the opinion that further work was necessary to establish the validity of DNA content as a measure of mammary development.

Experiments were designed to determine: (a) the applicability of a modified Schmidt and Thannhauser(6) procedure for nucleic acid extraction from mammary glands of rats, and (b) the DNA content per mammary cell nucleus during pregnancy and lactation.

Methods. Pregnant and lactating primiparous hooded Norway rats were used. Day 1 of pregnancy was the day sperm were noted in vaginal smears, and day 1 of lactation was the day of parturition. Litter size was adjusted to 8 pups per mother. To develop a nucleic acid procedure, rats pregnant 14, 18, or 20 days and rats lactating 12 or 14 days were used. In the DNA per nucleus experiment rats pregnant 12 or 20 days and rats lactating 1, 4, 8, 14, or 21 days were used.

Animals were sacrificed by cervical dislocation, and 6 abdominal-inguinal mammary glands were removed and washed quickly in ice-cold sucrose-bicarbonate buffer(7). After freezing on Dry Ice, glands were stored at –17°C until analyzed.

Tissue for nucleic acid determinations was defatted in a Bailey-Walker fat extraction apparatus, using 95% ethanol for 10 hours followed by anhydrous ether for 14 hours. After drying (6 hours at 37°C), the dried, ethanol-ether extracted tissue (DEEET) was weighed, frozen, and ground to a fine powder in a Wiley mill. Duplicate samples of 25 mg were extracted once with 5 ml of ethanol:chloroform (3:1, v:v). Samples were extracted twice with 5 ml of ice-cold 10% trichloroacetic acid (TCA), which was removed by washing the residue with ice-cold ethanol. The residue was digested in 3 ml of 1N KOH for 15 hours at 37°C. The digest was acidified with 0.4 ml ice-cold 6N HCl and 3.4 ml ice-cold 10% perchloric acid (PCA). The precipitate was washed twice with ice-cold 5% PCA. The combined supernatants were analyzed for ribose (RNA) by the colorimetric orcinol procedure(8). Mammary gland RNA content was calculated from a standard curve of highly purified yeast RNA.‡ DNA was extracted from

* Paper of the Journal Series, N. J. Agric. Exp. Station, Rutgers, The State University, New Brunswick.

† This study was supported by a grant from the Frelinghuysen Foundation.

‡ Kindly supplied by Dr. F. F. Davis, Dept. of Agric. Biochemistry, Rutgers, The State University.