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Effect of Dactinomycin on Estrogen-Induced Uterine Glycogen[1] (39803)

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Uterine glycogen is highest during proestrus, and exogenous estrogen administration elicits a deposition of the polysaccharide in the uterus (1). It has been well established that this polysaccharide is localized in the myometrium (2), and that the fluctuations in uterine glycogen during the estrous cycle correlate directly with alterations in estrogen secretion (3). However, the factors which influence estrogen-induced uterine glycogen are still not clear. Valadares *et al.* (4), using actinomycin D as an RNA inhibitor, concluded that estradiol influences glycogen synthesis independently of its effects on RNA synthesis. However, because RNA increases soon after estrogen stimulation and since RNA has been shown to control the increases in uterine wet weight, phospholipids, and protein synthesis due to estrogen (5, 6), it seems that the synthesis of this nucleic acid would be a prerequisite for the estrogen-induced increase in uterine glycogen.

Therefore, the purpose of this study was to determine whether or not RNA is necessary for the deposition of glycogen in the rat uterus following estrogen administration. This was done by treating the animals with estrogen and with dactinomycin ½ hr prior to estrogen stimulation and killing the animals, 2, 4, 6, and 8 hr following hormone treatment. RNA synthesis and uterine glycogen were analyzed at each of the time intervals.

Materials and methods. Young adult Holtzman rats, weighing 200–220 g, were housed three or four to a cage in an air-conditioned room maintained at 72°F, exposed to 14 hr of artificial light daily, and allowed free access to food and water. The rats were bilaterally ovariectomized 10 days before treatment. The 22 rats in Group I remained untreated, while the rats in Group II (23 rats) were each injected ip with 10 μg of estrone sulfate/0.1 ml of saline between 0900 and 0915 EST. The rats in Groups III (24 animals) and IV (23 animals) were each injected ip with 200 μg of dactinomycin[2] in saline between 0830 and 0845 EST. The rats in Group IV were also injected ip with 10 μg of estrone sulfate 30 min after dactinomycin administration.

All of the rats were injected with 25 μCi of tritiated [5-^{3}H]uridine (New England Nuclear; sp act = 39 Ci/mmole) 1 hr before necropsy. The animals were killed 2, 4, 6, or 8 hr after estrogen treatment. The uterine horns were excised, trimmed of extraneous tissue, and weighed to the nearest 0.1 mg. Weighed portions from both uterine horns were digested in boiling KOH; the amount of glycogen was determined by the indirect anthrone procedure (7). Portions of uterine horns from two or three rats were pooled and homogenized in 4 ml of cold distilled water with a mortar and pestle, and 1.5 ml of cold 20% TCA was added to the homogenate. Thirty minutes later the tubes were centrifuged, decanted, and the precipitate was washed twice in 5 ml of 5% cold TCA. The precipitate was resuspended in 3.0 ml of 0.2 *N* NaOH, boiled for 90 min, and 0.3 ml of 2 *N* HCl was added. Following precipitation by 5% TCA and centrifugation, an aliquot of the supernatant was analyzed for uridine uptake by liquid scintillation (8).

The statistical analysis was performed at the Bowman Gray Computer Center. The analysis of variance was determined and Duncan's multiple range test was used to locate significant differences.

Results. Uterine glycogen from rats killed 2 or 4 hr after estrogen stimulation was statistically similar to that from the un-

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[2] Dactinomycin was donated by Merck Sharp & Dohme.

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treated animals and the animals treated with dactinomycin and with estrogen plus dactinomycin. However, there was a significant increase ($P < 0.01$) in glycogen in the groups that were killed 6 or 8 hr after estrogen treatment as compared to any of the other three groups (Fig. 1). In the rats that were killed 8 hr after treatment with estrogen plus dactinomycin, the glycogen concentration was significantly more ($P < 0.05$) than that in the controls and in the rats treated with dactinomycin alone. All other comparisons between treatments and at different time intervals were not different ($P > 0.05$).

Although RNA synthesis from uteri of rats injected with estrogen was increased over control values at all time periods, a significant increase ($P < 0.01$) was observed only in the rats killed 2 hr after hormone treatment (Fig. 2). In all the other groups, regardless of time interval, RNA synthesis was lower but not significantly different from the controls.

Discussion. The results show that the increase in uterine glycogen following estrogen is preceded by an increase in RNA synthesis. Furthermore, when the increase in estrogen-induced RNA is inhibited by dactinomycin, there is also a significant suppression in estrogen-induced uterine glycogen. The results indicate that RNA synthesis has a major role in the deposition of uterine glycogen following estrogen stimulation. It is not clear why a significant increase in uterine glycogen in the estrogen-plus-dactinomycin group was observed 8 hr following treatment. This may be due, however, to a lack of complete suppression of RNA synthesis by the antibiotic. Other factors may account for the increase, such as glucose availability or activation of preexisting glycogenic enzymes.

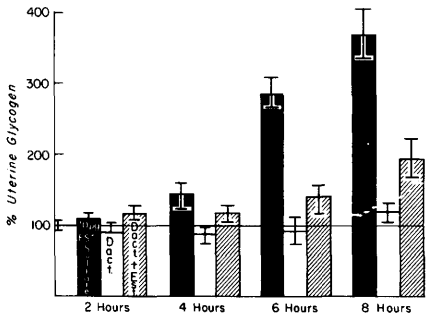

FIG. 1. Effects of estrone sulfate, dactinomycin, and dactinomycin plus estrogen on uterine glycogen at 2, 4, 6, and 8 hr after hormone treatment; the antibiotic was administered 30 min prior to estrogen. Each bar represents the percentage difference (±SE) from the control value of 100 ± 7%. At 6 and 8 hr the estrogen group was significantly different from the other three groups ($P < 0.01$). At 8 hr the dactinomycin-plus-estrogen group was significantly different ($P < 0.05$) from the control and dactinomycin groups. All other comparisons between treatments were not statistically different ($P > 0.05$). Each bar represents five to seven rats.

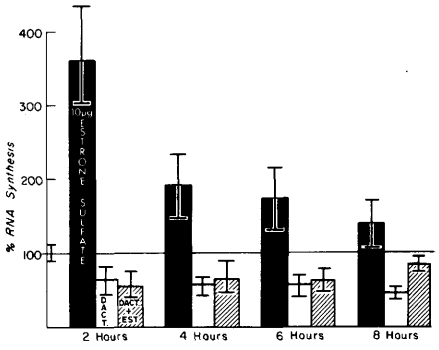

FIG. 2. Effect of estrone sulfate, dactinomycin, and dactinomycin plus estrogen on uterine RNA synthesis at 2, 4, 6, and 8 hr after hormone treatment; the antibiotic was administered 30 min prior to estrogen. Each bar represents the percentage difference (±SE) from the control value of 100 ± 19%. At 2 hr the estrogen group was statistically different ($P < 0.01$) from the other three groups. The other comparisons between treatments for each time period were not statistically different ($P > 0.05$). Each bar represents two or three determinations of pooled uteri from two or three rats.

The present conclusion that RNA synthesis has a major role in the deposition of uterine glycogen following estrogen stimulation differs from that of Valadares *et al.* (4), who concluded that estradiol influences the synthesis of glycogen in a manner which does not depend on RNA synthesis. However, in their study, the rats were killed 36 hr after treatment with actinomycin D and RNA synthesis was not determined.

The concept that a steroid hormone stimulates glycogen deposition in a target organ via RNA is not a new one. From studies on testosterone-induced glycogen of the rat prostate and seminal vesicle, Singhal *et al.*

(9) concluded that the hormone accelerates glycogenesis by stimulating a mechanism which is dependent on prior stimulation of RNA. Other reports have indicated that RNA synthesis, through its influence on enzymes, may be involved with the deposition of uterine glycogen following estrogen. Our earlier studies (10) suggest that the polysaccharide concentration in the uterus following estrogen stimulation is governed by the activities of glycogen synthetase and phosphorylase. Demers and Jacobs (11) considered these enzymes cardinal to the regulation of glycogen turnover in the rat uterus.

Summary. To determine whether or not RNA synthesis has a role in uterine glycogen deposition due to estrogen stimulation, ovariectomized rats were injected with either dactinomycin, estrogen, or estrogen plus dactinomycin. The antibiotic was administered 30 min prior to estrogen and the rats were killed 2, 4, 6, and 8 hr after hormone treatment. The results show that uterine glycogen is significantly increased at 6 and 8 hr, preceded by an increase in RNA synthesis. The estrogen-induced uterine glycogen is significantly suppressed by the antibiotic at 6 and 8 hr after the initial hormone treatment. The data indicate that RNA has a major role in the deposition of estrogen-induced uterine glycogen.

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