Amount and Distribution of Amylophosphorylase in Uterus of the Rat.* (24760)

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The amount and distribution of glycogen in component tissues of the uterus have been determined in a number of species and under a variety of physiological conditions. For the rat, histochemical studies have demonstrated that glycogen is present principally in smooth muscle of the myometrium and to a lesser extent in the lumenal epithelium of the endometrium(1,2). Among steroid sex hormones, estrogens and testosterone promote formation of glycogen whereas progesterone does not affect glycogenesis(2,3,4,5). Despite the considerable literature on metabolism of glycogen in the uterus, very little is known about the activity of enzymes concerned with synthesis and utilization of glycogen in this organ. Goldberg, *et al.*(6), employing a histochemical procedure that probably demonstrated combined actions of amylophosphorylase and amylo, 1-4→1-6 transglucosidase, reported abundant polysaccharide formation in uterine musculature of human and rabbit and variable activity in endometrium of the former. Takeuchi, *et al.*(7), using a histochemical method more or less specific for amylophosphorylase, found intense activity in uterine muscle of dog, horse and human, but reported that the enzyme was "not readily demonstrable" in uteri of rat and rabbit. The present investigation was undertaken to determine quantitatively the activity of amylophosphorylase in the uterus of rats treated with steroid sex hormones and to localize the enzyme by histochemical means.

Materials and methods. Eighty-five young adult female albino rats were used. The animals were bilaterally ovariectomized. Uterine phosphorylase was determined quantitatively in 63 rats as follows: One group of 10 controls received no further treatment. Remaining animals were divided into 5 groups of 8 to 12, injected subcutaneously on each of 3 consecutive days with either (1) 0.1, (2) 0.5, (3) 1.0 μg estradiol benzoate, (4) 0.5 μg estradiol benzoate and 1 mg progesterone concurrently, or (5) 1 mg progesterone alone. Crystalline hormones were used, daily dose administered in 0.05 to 0.1 ml of sesame oil. Twenty-four hours after last injection the animals were killed with chloroform, as were the uninjected controls. Uteri were removed and one horn was weighed to nearest 0.1 mg with Roller-Smith torsion balance, then homogenized in chilled Potter-Elvehjem apparatus. The contralateral horn from each uterus was also weighed, then dried under infrared lamp to determine amount of dry tissue residue. Enzyme activity was assayed in duplicate in 0.2 ml aliquots of a 10% suspension of homogenized fresh tissue in 0.1 M NaF following the procedure of Sutherland and Wosilait(8) with minor modifications. This method utilizes rate of liberation of inorganic P from glucose-1-phosphate as the measure of phosphorylase activity. The results are expressed as 0.01 mg of inorganic P liberated/mg of dry tissue/10 minutes incubation at 37°C. Localization of enzyme activity was determined histochemically in the remaining 22 rats as follows: Frozen sections, 25 to 50 μ thick, were cut on clinical microtome from uteri of both untreated castrates and animals receiving various amounts of estradiol benzoate as indicated above. Freshly-cut sections were then incubated 30 minutes to 3 hours at 37°C in the following mixture which was adapted from methods of previous workers (6,7,9):

glucose-1-phosphate	50	mg
muscle adenylic acid	10	"
glycogen	50	"
distilled water	7.5	ml
saturated Na acetate sol.	2.5	"
insulin	8.0	units

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TABLE I. Uterine Phosphorylase Activity in Sex Hormone-Treated Ovariectomized Rats.

Treatment*	No. of animals	Phosphorylase activity† (mean ± S.E.)
None	10	2.31 ± .16
.1 μg estradiol benzoate	12	2.21 ± .22
.5 μg *Idem*	12	2.02 ± .15
1.0 μg "	9	1.79 ± .22
.5 μg estradiol benzoate and 1.0 mg progesterone	8	2.92 ± .27
1.0 mg progesterone	12	3.11 ± .32

* Hormones administered subcut. once daily for 3 days.

† Expressed as 0.01 mg of inorganic P liberated per mg dry tissue/10 min. incubation at 37°C.

The mixture was adjusted to pH of 5.5 with 0.1N HCl. After incubation the sections were rinsed with water, floated onto glass slides and air dried. Newly-formed glycogen in the sections was visualized in one of 2 ways. In most instances slides were placed 5 minutes in 70%, 95% and absolute alcohol, each containing 1% iodine and 2% KI. The sections were then cleared in xylene and mounted in routine manner. The glycogen was stained brick red to brown, the color gradually fading after several weeks. In some instances, after formation of glycogen-iodine complex in an aqueous solution of 1% iodine and 2% KI, the sections were treated with 1% silver nitrate followed by a photographic developer according to procedure of Lower (10) with minor modifications. This results in replacement of the red to brown glycogen-iodine complex with a permanent black precipitate of metalic silver. Control sections were prepared by procedures outlined above except that glucose-1-phosphate substrate was omitted from incubation mixture.

Results. The quantitative measurements of uterine phosphorylase activity are summarized in Table I. Statistical analysis indicates that there is no signicant difference in enzyme activity between any of the groups of hormone-treated rats and castrate controls. Histochemical studies revealed that in all animals, regardless of treatment, there was uniformly moderate to strong enzyme activity throughout the longitudinal and circular muscle of the myometrium and irregular and weak activity in the lining epithelium of the endometrium.

It is clear from these observations that the morphologic distribution of phosphorylase in the rat uterus is the same as that of uterine glycogen. Since enzyme activity of these tissues, unlike their glycogen content, is not affected by castration and estrogen treatment, the immediate mechanism involved in deposition of glycogen must be other than a change in enzyme activity.

Summary. Amylophosphorylase activity of the uterus was determined both quantitatively and histochemically in castrate rats and rats treated with estrogen and/or progesterone. There was no signicant difference in enzyme activity between any of the groups of animals regardless of treatment. Moderate to strong activity was uniformly present throughout the myometrium, whereas in the endometrium there was but irregular and weak activity in the lining epithelium.

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