Comparative Effects of Ovarian Steroids on Glycogen Metabolism of Rat, Rabbit and Guinea Pig Uterine Tissue (37491)

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The mammalian uterus has a unique potential for proliferative growth and differentiation which is cyclic. It is recognized that hormonal influences in the form of ovarian steroids generate the cyclicity of metabolism in uterine tissue which is predisposed to either the nidation process or to degeneration depending on whether conception has occurred. Among the many metabolic variables influenced by ovarian steroids in uterine tissue is the nature of glycogen metabolism. Several investigators have demonstrated in uterine tissue from the rat (1–5), mouse (6), hamster (7), guinea pig (8) and human (9, 10) that estrogen and progesterone play a definitive role in augmenting levels of glycogen. The heterogenous composition of this organ and species differences inherent in the hormone response make common interpretations of uterine control by ovarian hormones a meaningful and difficult task.

In the present study, comparative investigations were performed in the female rat, guinea pig and rabbit uterus to establish common effects of the ovarian steroids on uterine glycogen metabolism at the level of the enzyme systems considered cardinal to the regulation of glycogen turnover.

Materials and Methods. Animals. Young adult female animals (rats, rabbits and guinea pigs) were bilaterally ovariectomized for study. Rats were used for experimentation 10–14 days after the operation. Rabbits and guinea pigs were ovariectomized for 3–4 wk prior to experimental use.

Animals were injected subcutaneously with 5 μg estradiol-17β/100 g body weight at time zero and again 24, 48 and 72 hr later. Other animals received estradiol at time zero and 24 hr later followed by subcutaneous injections of 1 mg progesterone/100 g body weight for an additional 48 hr with injections given 24 hr apart. The steroid hormones were dissolved in sesame oil with this vehicle given to control animals.

Rats and guinea pigs were sacrificed by decapitation while rabbits were killed by a single blow on the head at 48 and 96 hr following the initial zero time estrogen injection.

The uteri from the rat and guinea pig animal groups were quickly excised, trimmed of extraneous tissue and adhering fat and immediately frozen in dry ice for subsequent glycogen content and enzyme activity measurements. The rabbit uteri upon removal were further prepared in the following manner. A portion of one uterine horn was removed for total uterine assay as described for the rat. The other horn was sliced longitudinally and the endometrial component scraped from the myometrium with the dull blade of a scalpel. Assays in the rabbit were performed on total uterus, myometrium and endometrium.

Biochemical Methods. Glycogen content determination. Tissue glycogen content in all studies was determined using a modification of the method described by Montgomery (11). The glycogen was precipitated from a 30% KOH tissue digest with alcohol and quantitated colorimetrically with a phenol–sulphuric acid mixture and known standard solutions.

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UDP-glucose:glycogen α4-glucosyl transferase (EC2.4.1.11) enzyme activity was quantitated by measuring the incorporation of ^{14}C-labeled groups from UDP-^{14}C glucose to glycogen with subsequent isolation of the radioactive polysaccharide as ^{14}C-labeled glycogen. This isotope technique was first described by Villar-Palasi and Larner (12) and modified kinetically to uterine tissue in our laboratories (13). Glycogen transferase activity was determined in the presence (total) and absence of the cofactor metabolite G-6-P. The reaction was initiated by the addition of 100 μl of a low-speed uterine homogenate supernatant (5 mg tissue) prepared in 0.25 *M* sucrose containing 10 m*M* NAF and 1.7 m*M* EDTA to the following reaction mixture: 40 m*M* tris-Maleate buffer pH 7.5, 1.7 m*M* EDTA, 0.48% glycogen primer, 32 m*M* G-6-P (when required), 2.4 m*M* UDPG, 0.03 μCi UDP-μl-^{14}C glucose (specific activity 147 mCi/mmole) in a total volume of 0.3 ml. Incubations were carried out for 15 min at 37° and the reaction terminated with 1 ml 30% KOH containing 1 mg LiBr and 1 mg glycogen as carrier. The above mixture was boiled and the glycogen isolated by the addition of 2 ml 95% ethanol. The precipitated glycogen was washed once with distilled water and the reprecipitated glycogen subjected to radioactive counting to estimate the amount of ^{14}C label incorporated into glycogen. Control blanks were processed along with all unknown specimens and were obtained by adding the 30% KOH stop reagent prior to the addition of tissue supernate.

Radioactive counting was performed in an ambient temperature Beckman (Model LH 150) liquid scintillation counter with automatic quench correction present for a ^{14}C-dioxane system. The liquid scintillation cocktail consisted of 1.3% hydroxide of hyamine; 0.6% napthalene; 0.4% 2,5-diphenyl oxazole; 0.20% 1,4-bis[*z*-(4 methyl-5-phenyl-oxazolyl]-benzene all in a dioxane base of 15 ml total volume. Under the conditions of the assay, an enzyme unit corresponds to the incorporation of 1 μmole of radioactive glucose into glycogen per gram of wet tissue per hour.

Glycogen phosphorylase activity. The assay of glycogen phosphorylase was determined according to Demers (13) based on reverse glycogenolysis and featured the incorporation of labeled ^{14}C glucose into glycogen from ^{14}C glucose-1-phosphate. Enzyme activity was determined in the presence and absence of the cofactor 5′-adenosine monophosphate. The reaction mixture as used contained 100 μl of low-speed (350*g*) supernatant from a 5% uterine sucrose homogenate containing 10 m*M* NAF and 1.7 m*M* EDTA, 26 m*M* G-1-P, 0.42% glycogen, 9.0 m*M* 5′AMP (when required), 50 m*M* tris-Maleate buffer pH 6.1, 0.01 *M* NAF and 0.05 μCi glucose-1-phosphate (specific activity, 200 mCi/m*M*) in 0.3 ml total volume. Incubations were performed at 37° for 10 min and the reaction terminated with 1 ml 30% KOH with carrier glycogen (1 mg/ml). The radioactive polysaccharide was isolated and processed for counting in a manner similar to that described for the synthetase enzyme. Under established controlled kinetic conditions, an *enzyme unit* was equal to 1 μmole of radioactive glucose incorporated into glycogen per gram wet tissue per hour.

Results. The administration of estradiol to adult ovariectomized female rats, rabbits and guinea pigs brought about a similar and marked elevation in uterine glycogenesis. Forty-eight hours following the initiation of estradiol treatment (Table I), the uterine glycogen content of the rat increased to 230% of the control value, the rabbit showed a 278% increase and the guinea pig a 247% increase. These increases in uterine glycogen content were accompanied by a corresponding rise in the uterine glycogen synthetase enzyme levels with the rat and guinea pig having the greatest relative increases. Some change in the activity of the uterine phosphorylase enzyme system was observed after the 48-hr period of estrogen treatment. This was statistically significant for the phosphorylase form (a) active in the absence of the cofactor 5′AMP in the rats and rabbits. The guinea pig uterus showed evidence of both forms of phosphorylase enzyme activity elevations after the estradiol treatment of 48 hr.

After a period of 96 hr of estrogen, the glycogen content in all three species was de-

TABLE I. Effect of Ovarian Steroids on Uterine Glycogen Metabolism in Rat, Rabbit and Guinea Pig.

Treatment	Post injection (hr)	Animal	Glycogen content (mg/100 g)	Glycogen synthetase (μmoles glucose/g/hr) +G-6-P	Glycogen phosphorylase (μmoles glucose inc./g/hr) +AMP	Glycogen phosphorylase (μmoles glucose inc./g/hr) —AMP
Control	0	Rat	154 ± 11	14.3 ± 0.21	1060 ± 26	786 ± 27
Estradiol[a]	48		355 ± 22*	36.3 ± 1.03*	1119 ± 24	908 ± 16*
Estradiol	96		278 ± 19*	44.0 ± 1.23*	1377 ± 34*	1145 ± 30*
Control	0	Rabbit	165 ± 16	26.2 ± 0.65	286 ± 13	157 ± 8
Estradiol	48		459 ± 34*	38.3 ± 1.34*	273 ± 15	188 ± 11*
Estradiol	96		265 ± 27*	45.2 ± 1.53	452 ± 26*	253 ± 13*
Control	0	Guinea pig	74 ± 8	50.2 ± 1.3	618 ± 30	417 ± 19
Estradiol	48		257 ± 21*	73.4 ± 1.4*	861 ± 24*	484 ± 16
Estradiol	96		168 ± 11*	89.6 ± 1.2*	888 ± 30*	692 ± 17*
Progesterone[b]	48	Rat	225 ± 17*	25.4 ± 0.58*	988 ± 29	750 ± 20*
Progesterone	48	Rabbit	272 ± 14*	55.3 ± 1.5*	473 ± 13	438 ± 18*
Progesterone	48	Guinea pig	123 ± 14*	94.7 ± 1.7*	1057 ± 28*	979 ± 16*

[a] Injected subcutaneously with 5 μg estradiol-17β/100 g body weight at time zero and again at 24, 48 and 72 hr later.

[b] Injected subcutaneously with 1 mg progesterone/100 g body weight at 0 time and 24 hr later following a 48-hr estrogen prime. Zero time was considered as 48 hr following the initial estrogen injection of 5 μg E_2/100 g body weight. Control animals received sesame oil vehicle injections at each time interval. Results expressed as mean ± SE.

* Statistically different at $p < 0.01$ level as compared to control. Each group consisted of five animals.

creased from the 48-hr treatment level; however, the total synthetase enzyme continued to show increased activity at this time. Both phosphorylase enzyme activities were elevated in all three species of animals at this time period of estrogen treatment. The increased phosphorylase activity is probably reflected in the glycogen content decline observed at 96 hr post estrogen treatment when a steady state has developed between the two enzyme systems controlling glycogen turnover.

When progesterone was administered following a priming period of 48 hr with estradiol, there were different effects observed which appeared to be species related. The rat uterus appeared unaffected by progesterone treatment with consistent decreases in glycogen content, synthetase enzyme and phosphorylase enzyme activity levels found following withdrawal of estrogen. The rabbit and guinea pig, on the other hand, gave a definite uterine response to progesterone; this was particularly evident with the phosphorylase enzyme activity levels. The total uterine glycogen content declined in both the rabbit and guinea pig from the maximal level observed at 48 hr (Table I) even though the synthetase enzyme showed slight activity increases. Both the rabbit and guinea pig exhibited a progestational type uterine response when administered the progesterone following an estrogen prime. This was most pronounced in the rabbits where enormous endometrial growth was apparent. The rabbit uterus in response to progesterone had almost a twofold increase in the active phosphorylase from (—AMP) while the guinea pig showed significant changes in both forms of this enzyme system.

The results of ovarian steroid treatment on glycogen metabolism in the individual uterine components (myometrium and endometrium) of the rabbit are shown in Table II. Estrogen treatment caused a glycogenic response primarily in the myometrial portion of the uterus which is best reflected by the

TABLE II. Effect of Ovarian Steroids on Glycogen Turnover in Rabbit Endometrium and Myometrium.

Treatment[a]	Post injection (hr)	Component	Glycogen content (mg/100 g)	Glycogen synthetase (μmoles glucose/g/hr) +G-6-P	Glycogen phosphorylase (μmoles glucose inc./g/hr) +AMP	Glycogen phosphorylase (μmoles glucose inc./g/hr) —AMP
Control (5)	0	Uterus	165 ± 16	26.2 ± 0.65	286 ± 13	157 ± 8
		Myometrium	168 ± 21	26.6 ± 0.70	263 ± 11	143 ± 9
		Endometrium	143 ± 13	24.5 ± 0.68	230 ± 22	185 ± 13
Estradiol (5)	48	Uterus	459 ± 34*	38.3 ± 1.34*	273 ± 15	188 ± 11*
		Myometrium	463 ± 43*	43.1 ± 2.26*	276 ± 15	195 ± 13*
		Endometrium	154 ± 17	33.9 ± 0.26*	254 ± 19	227 ± 16*
Estradiol (5)	96	Uterus	265 ± 27*	45.2 ± 1.53*	452 ± 26*	253 ± 13*
		Myometrium	335 ± 29*	44.2 ± 1.65*	473 ± 27*	312 ± 18*
		Endometrium	138 ± 11	26.2 ± 1.13	256 ± 15	227 ± 11*
Progesterone (5)	48	Uterus	272 ± 14	55.3 ± 1.5*	473 ± 13*	438 ± 18*
		Myometrium	174 ± 11	32.1 ± 2.6	463 ± 24*	443 ± 17*
		Endometrium	283 ± 26	43.9 ± 1.6	291 ± 9*	276 ± 11*

[a] Estrogen and progesterone administered as indicated in legend on Table I.

* Statistically significant at $p < 0.01$ when compared to control value (Student t test). Results expressed as mean ± SE. Number in parentheses refers to five animals.

significant increases noted after 48 hr post estrogen treatment in both myometrial glycogen content and myometrial glycogen synthetase activity. Although total (+AMP) phosphorylase activity in the myometrium was unaffected by the estrogen treatment, the active form (—AMP) of the enzyme was elevated at the 48-hr treatment period.

Following 96 hr of estrogen treatment to ovariectomized rabbits, total myometrial synthetase activity remains elevated but in addition both the total and active forms of phosphorylase in the myometrium are increased. The elevated phosphorylase activity to estrogen at 96 hr is most probably responsible for the decreased myometrial glycogen found at this time period when compared to the 48-hr peak concentrations.

It is interesting to note that estrogen treatment alone was essentially without influence on endometrial glycogen metabolism. In contrast, the glycogen content and total glycogen synthetase activity of rabbit endometrial tissue was significantly elevated in response to progesterone treatment following a 48 hr priming period of estrogen (Table II). The phosphorylase enzyme activity (both forms) in rabbit endometrium was only slightly elevated following progesterone treatment.

Discussion. In recent years, there have been numerous reports attempting to elucidate the mechanisms whereby the ovarian steroids exert regulatory control over certain uterine metabolic pathways by direct influence on the synthesis and degradation of select key rate-limiting enzyme systems (15–17). A clear understanding of the hormonal regulation of metabolic events within the uterine cell has been complicated by the lack of homogeneity of cell types within this organ and to differences in hormone response which appear to be species specific. Most studies reported deal with uterine responses in general and fail to differentiate between events which take place in the component parts of the uterus.

The endometrial and myometrial components of the uterus are so remarkably well differentiated with respect to both structure and function that any biochemical study of uterine metabolism and physiology must clearly distinguish between them. Differences in activity between uterine tissue components have been demonstrated for carbonic anhy-

drase by Lutwak-Mann and Adams (18), for succinic acid oxidase by Telfer and Hisaw (19) and for alkaline phosphatase by Giering and Zarrow (20).

Recently, Yochim and his collaborators (21, 22) have attempted to differentiate between the myometrium and endometrium of the rat in studies relating to progesterone differentiation during progestation. They noted in several instances particularly with respect to uterine lactic dehydrogenase activity, significant differences in isozyme distribution between the rat endometrium and myometrium.

In the studies reported here, estrogen replacement following ovariectomy elicited similar changes in all three species studied with respect to estrogen induced uterine glycogenesis. Although the enzyme activity levels varied between species, the direction of the hormone response was similar with estrogen treatment. The administration of progesterone, on the other hand, evoked dissimilar changes in the glycogen metabolizing enzymes which appear to be species related. Progesterone treatment to the estrogen-primed ovariectomized rat was without influence on any of the parameters of uterine glycogen metabolism studied. On the other hand, progesterone evoked synergistic changes in both the rabbit and guinea pig uterine tissue in terms of the activity levels of glycogen synthetase and phosphorylase.

The guinea pig uterine glycogen metabolic response to progesterone was similar to that observed with the rabbits, although the glycogen decline was more pronounced and there was almost a two-fold increase in the active form of phosphorylase.

Studies on the individual uterine tissues in the rabbit uterus clearly demonstrate differences in hormone response with respect to the glycogen metabolizing enzymes. The biochemical changes produced by estrogen treatment of the ovariectomized rabbit reflected primarily changes in myometrial metabolism. This is understandable since the myometrium comprises by far the major component of this tissue. Progesterone treatment produced changes which were indicative of increased endometrial metabolism, an increase in glycogen content of the endometrium and a correlated elevation in endometrial glycogen synthetase activity. This finding is in agreement with *in vitro* organ culture studies with human endometrium (10).

Although further studies are required to define how the individual ovarian steroids interact with the component parts of the uterus, the present findings suggest that during the course of hormone-induced uterine growth and proliferation in the rabbit and guinea pig, myometrial glycogenesis is primarily influenced by estrogen while endometrial glycogen deposition appears to be a function of progesterone. These differences in hormone response to the endometrium and myometrium in terms of glycogen metabolism may well be due to the hormone receptor concentration differences of the individual steroids that exist in different endocrine states. It is well documented that the progestational response, both morphologically and biochemically to progesterone in any species, occurs only when an estrogen prime is given.

Summary. The effects of the ovarian steroids, estrogen and progesterone on uterine glycogen metabolism in the rat, guinea pig and rabbit were compared. Estrogen caused a uterine glycogenic response in all three species which was maximal, 48 hr following the initiation of treatment to adult ovariectomized females. Progesterone treatment to these animals following a 48-hr estrogen prime elicited a significant increase in uterine phosphorylase activity in the guinea pig and rabbit but had no effect on rat uterine phosphorylase. Studies on the individual components of the rabbit uterus (myometrium and endometrium) in response to estrogen and progesterone revealed different effects in uterine glycogen metabolism. The myometrium was more responsive to estrogen while the endometrium was more responsive to progesterone in terms of uterine glycogenesis. These findings suggest that different cell types within the uterus respond differently to different steroids depending on the endocrine state of the animal.

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Received Feb. 23, 1973. P.S.E.B.M., 1973, Vol. 143.