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Comparison of UDPG-Glycogen Synthetase Activity of the Tongue and Uterus.* (28498)

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The two enzymes, phosphorylase and uridinediphosphoglucose (UDPG)-glycogen synthetase, synthesize 1,4 linkages of glycogen with glucose-1-phosphate (G-1-P) and UDPG as their respective substrates. The action of phosphorylase is readily reversible so that this enzyme may be involved in both synthesis and breakdown of glycogen while UDPG-glycogen synthetase is concerned only with synthesis of glycogen. According to Stetten and Stetten(1) there seems to be conclusive evidence that in most tissues the synthesis of glycogen proceeds largely through the action of UDPG-glycogen synthetase and the branching enzyme, while the function of phosphorylase in conjunction with that of a debranching enzyme (amylo-1,6-glucosidase) is involved in the breakdown of glycogen to G-1-P.

Glycogen synthesized from UDPG cannot be demonstrated histochemically in the uterus of the rat and rabbit while glycogen synthesized from G-1-P can be demonstrated in the myometrium of these two species(2). However, glycogen synthesized from UDPG and G-1-P can be observed histochemically in the tongue(2).

The present investigation was designed to determine whether UDPG-glycogen synthetase is present in the uterus of the rat and, if present, how its activity is altered by the ovarian hormones. The investigation was also designed to compare UDPG-glycogen synthetase activity of skeletal muscle of the tongue and smooth muscle of the uterus.

Materials and methods. Thirty-nine young adult Wistar rats (Charles River) were maintained on Purina Chow and water *ad libitum* throughout the experiment. All rats were bilaterally ovariectomized and 10 days following ovariectomy 9 animals were sacrificed without further treatment. The remaining rats were divided into groups receiving the following daily hormonal† treatment: I. 1 μg of estrogen (10 animals); II. 1 mg of progesterone (10 animals); III. 1 μg of estrogen plus 1 mg of progesterone (10 animals). The hormones were injected sub-

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TABLE I. Comparison of UDPG-Glycogen Synthetase Activity Between the Tongue and the Uterus of the Rat.*

Organ or treatment	No. anmials	Net specific activity†	P
Uterus			
Control	9	.001 ± .003‡	
Estrogen	10	.001 ± .001	>.9
Progesterone	10	.000 ± .002	>.4
Estrogen and progesterone	10	.002 ± .003	>.4
Tongue	14	.015 ± .006	<.001

* All rats were bilaterally ovariectomized 10 days prior to hormone treatment.

† Activity of enzyme = μmoles UDP formed/ml homogenate/min. Specific activity = activity/mg protein/ml homogenate.

‡ Mean ± standard deviation.

cutaneously on 3 consecutive days and the animals were killed with ether 24 hours after the last injection.

At autopsy a portion of the uterus was removed, blotted and weighed. The uterus was then placed in cold 0.25 M sucrose solution containing 0.001 M ethylenediaminetetraacetic acid and homogenized with a mortar and pestle containing sand. Also, at autopsy, a portion of the tongue was removed and treated in a similar manner as was the uterus. The procedure of Leloir and Goldenberg(3) was followed to determine UDPG-glycogen synthetase activity.

The activity of glycogen synthetase was expressed in μmoles of UDP formed/ml of homogenate/min. The micro-Kjeldahl method was used to determine nitrogen and protein content calculated by multiplying the amount of nitrogen by 6.25. Specific activity of the enzyme was expressed by the ratio: activity/mg protein/ml homogenate.

Observations. The results obtained demonstrate that there was no significant increase in UDPG-glycogen synthetase activity of the uterus between hormone-treated animals and ovariectomized animals. However, a significant increase in the enzyme was observed between the tongue and uterus of the ovariectomized rats (Table I).

In comparing the experimental reaction mixture (containing UDPG) with the control reaction mixture (lacking UDPG) a significant increase in activity of the enzyme was observed in the tongue, ($P<0.001$). A significant increase in enzymatic activity was not present in the uteri of ovariectomized or ovariectomized hormone-treated rats. Statistical analysis showed that in the ovariectomized and progesterone-treated rats $P = >0.9$. The estrogen-treated animals and the estrogen- and progesterone-treated animals had P values of >0.6 and >0.5 respectively.

Discussion. These results demonstrate that UDPG-glycogen synthetase activity is not present in the uterus of ovariectomized or ovariectomized hormone-treated rats. However, the enzyme is present in the tongue of these animals.

The question of uniformity of metabolic pathways has recently been discussed by Bueding(4) who points out that the overemphasis of the concept of uniformity of pathways has hidden to some degree the problem of biochemical variabilities and indicates that a difference exists in analogous enzymes such as glycogen phosphorylases of the liver and skeletal muscle. Phosphorylase activation in skeletal muscle requires 5-adenylic acid while that of liver phosphorylase does not. Like the observations on lactic dehydrogenases by Kaplan and co-workers(5), Bueding(4) observed that there are greater similarities among phosphorylases of the same tissue in different species than between 2 different tissues, smooth and skeletal muscle, of the same species. Recent histochemical studies have shown that a difference exists in the phosphorylase activity and UDPG-glycogen synthetase activity of muscle fibers(6). In fibers which have a large diameter phosphorylase activity was very strong whereas in fibers of small diameter (red type) a very high activity of UDPG-glycogen synthetase was observed.

Glycogen synthesized from G-1-P can be demonstrated in the smooth muscle cells of the uterus and the skeletal muscle cells of the tongue while glycogen synthesized from UDPG can be demonstrated only in the skeletal muscle cells of the tongue(2). The present results show that there is a variability in glycogen synthesis between tissues, in agreement with the observations of Bueding

(4). In the uterus of the rat glycogen synthesis is catalyzed by phosphorylase from G-1-P and not through the UDPG pathway. Since the morphology and physiology of smooth muscle and skeletal muscle are not the same the difference in the metabolic pathway for synthesis of glycogen is not surprising. It is possible that smooth muscle cells of organs that are not stimulated by ovarian hormones can behave in a different way from the smooth muscle cells of the uterus.

Summary. UDPG-glycogen synthetase was not demonstrated in the uterus of the ovariectomized or in the ovariectomized-hormone treated rats. However, in the tongue the enzyme was present. The results show that glycogen of the rat uterus is not synthesized through the UDPG pathway. The results also demonstrate a difference in glycogen synthesis between the smooth muscle of the uterus and skeletal muscle of the tongue.

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Role of Hepatic L-α-Glycerophosphate and Triglyceride Synthesis in Production of Fatty Liver by Ethanol.* (28499)

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Ethanol is known to interfere with lipid metabolism in such a way that a prompt increase in the triglyceride content of the liver follows a large single dose(1). A more prolonged ethanol administration causes, in addition, a hypertriglyceridemia in animals(2) and man(3,4).

The mechanism of these metabolic alterations has not been definitely established. Evidence for an increased mobilization of fat from depots after ethanol has been presented by Horning *et al.*(5) in demonstrating that the fatty acid composition of liver triglycerides of ethanol-treated rats resembles that of adipose tissue. In further support of this concept is the elevated level of plasma free fatty acids reported to occur after intoxicating doses of ethanol in rat(6,7). However, others have not been able to confirm the last finding, and have therefore not accepted the fat mobilization theory(8,9). On the other hand, Lieber and Schmid(10) have demonstrated that ethanol *in vivo* and *in vitro* increases the synthesis of fatty acids and inhibits their oxidation in rat liver slices. Similar results were obtained also by Reboucas and Isselbacher(11), who showed, however, that these effects of ethanol do not adequately explain the development of fatty liver. Ethanol has also been reported to increase the incorporation of palmitic acid into triglycerides *in vitro* by cell-free homogenates of rat liver(12), and, in a perfusion experiment, to decrease the release of triglyceride from the liver(13).

In addition to the above mechanism there remains the possibility that ethanol could increase the rate of hepatic uptake of fatty acids from circulation and their esterification with α-glycerophosphate, *i.e.*, the synthesis of triglycerides. This point has been investi-

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