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A Histochemical Comparison of Glycogen Synthesis from Glucose-1-Phosphate and Uridinediphosphoglucose in Uterine Sections.* (27740)

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In vitro, synthesis of glycogen-like polysaccharides is easily obtained by the combined action of amylo-1,4-1,6 transglucosidase with either a phosphorylase or uridinediphosphoglucose (UDPG)-glycogen transferase. The latter 2 enzymes synthesizing 1,4 linkages with glucose-1-phosphate (G-1-P) and UDPG as their respective substrates. Unlike UDPG-glycogen transferase the action of phosphorylase is readily reversible, so that this enzyme may be involved in both synthesis and breakdown of glycogen. However, there seems to be conclusive evidence that in most tissues the synthesis of glycogen proceeds largely through the action of UDPG-glycogen transferase and of the branching enzyme, while the function of phosphorylase in conjunction with that of a debranching enzyme (amylo-1,6-glucosidase) being chiefly involved in the breakdown of glycogen to G-1-P,(1).

Takeuchi and Kuriaki(2) and Takeuchi and Glenner(3) were able to demonstrate histochemically the presence of amylophosphorylase and UDPG-glycogen transferase by incubating tissue slices with G-1-P or UDPG respectively and staining the incubated tissues with iodine.

It has previously been demonstrated histochemically that in the uteri of rats and rabbits phosphorylase activity is confined to the myometrium and that the activity of the enzyme was at its highest following estrogen stimulation(4,5). This investigation, in addition to confirming and extending my previous findings, was designed to determine whether glycogen synthesis from UDPG can be demonstrated in uterine sections of the ovariectomized, ovariectomized-hormone treated rats and rabbits and how the activity of UDPG-glycogen transferase, if present, is altered by the ovarian hormones.

Materials and methods. Twenty-three adult virgin female rabbits were used in this study. All the animals were bilaterally ovariectomized. Three weeks following ovariectomy 5 rabbits were killed without further treatment. The remaining animals were divided into groups and received the following daily hormone† treatment: I. 10 μg of estrogen (6 animals); II. 2.5 mg progesterone (6 animals); III. 10 μg estrogen plus 2.5 mg progesterone (6 animals). In Groups I and II the hormones were injected subcutaneously on 7 consecutive days while in Group III estrogen was injected for 7 consecutive days followed by injection of progesterone for 7 consecutive

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TABLE I. Distribution of Phosphorylase and UDPG-Glycogen Transferase in Uteri of Hormone Treated Ovaricctomized Rats.

Condition or treatment	No. animals	Myometrium		Endometrium (epithelium)	
		Phosphorylase	UDPG-glycogen transferase	Phosphorylase	UDPG-glycogen transferase
Control	4	+	0	0	0
Estrogen	8	+++	0	++	0
Progesterone	6	+	0	0	0
Estrogen and progesterone	6	++	0	0	0

Section of the tongue and heart demonstrated phosphorylase and UDPG-glycogen transferase activity.

TABLE II. Distribution of Phosphorylase and UDPG-Glycogen Transferase in Uteri of Hormone Treated Ovariectomized Rabbits.

Condition or treatment	No. animals	Myometrium		Endometrium (epithelium)	
		Phosphorylase	UDPG-glycogen transferase	Phosphorylase	UDPG-glycogen transferase
Control	5	+	0	0	0
Estrogen	6	+++	0	0	0
Progesterone	6	±	0	0	0
Estrogen and progesterone	6	+	0	0	0

Section of the tongue and heart demonstrated phosphorylase and UDPG-glycogen transferase activity.

days. The animals were autopsied 24 hours after the final injection. At autopsy a portion of the tongue, heart and uterus was removed and placed on dry ice. The details of staining are given below.

Twenty-two rats of the Wistar strain (Charles River) were used in this experiment. All the animals were bilaterally ovariectomized. After 10 days, 4 animals were sacrificed without further treatment. The remaining rats were divided into groups receiving the following daily hormonal treatment: I. 2 μg of estrogen (8 animals); II. 1 mg progesterone (6 animals) and III. 2 μg estrogen plus 1 mg progesterone concurrently (6 animals). The hormones were injected on 3 consecutive days and the animals autopsied 24 hours later. At autopsy a portion of tongue, heart and uterus was placed on dry ice.

The frozen sections of tongue, heart and uterus from both the rabbit and rat were treated in a similar manner. Frozen sections were made on the cryostat at 16 μ and then placed in the substrates. The incubating media of Takeuchi and Kuriaki(2) for demonstrating phosphorylase activity consisted of 50 mg G-1-P, 10 μg muscle adenylic acid; 15 ml distilled water; 10 ml 0.2 M acetate buffer at ph 5.8; 15 units of insulin and 5 mg glycogen. The sections were incubated for one hour at 37°C. The control sections were placed in a similar incubating solution lacking G-1-P. Sections were removed from the incubating media after one hour and placed in dilute Lugol's solution (5 ml Lugol's solution USP in 95 ml distilled water) (I_2:KI:H_2O; 1:2:400).

Also, frozen sections of the tissues mentioned above were placed in the substrate of Takeuchi and Glenner(3) to demonstrate UDPG-glycogen transferase activity. The substrate consisted of 50 mg UDPG; 10 mg glycogen; 20 mg Versene; 10 mg G-6-P; 14 ml distilled water; 10 ml 0.2 M tris-buffer (ph 7.4) and 1 ml absolute alcohol. The sections were incubated for one hour at 37°C. The control sections were placed in the same substrate lacking UDPG. The sections were removed from the substrate and placed in Gram's solution (I:KI:H_2O; 1:2:300) which was diluted about 10 times with distilled water.

Observations. The results obtained are summarized in the Tables. In sections of the

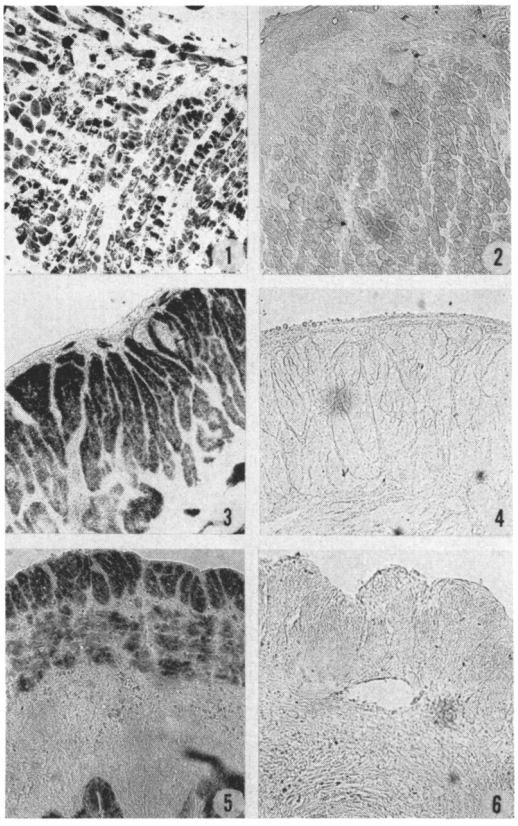

FIG. 1. Section of the tongue placed in substrate containing UDPG and then placed in Gram's solution. Black color demonstrates UDPG-glycogen transferase. ×50.

FIG. 2. Section of tongue incubated in substrate lacking UDPG and stained in Gram's solution. Black color is absent. ×50.

FIG. 3. Section of rabbit uterus incubated in substrate containing glucose-1-phosphate and stained with Lugol's solution. Black color indicates phosphorylase activity which is in the myometrium. Animal was treated with estrogen. ×50.

FIG. 4. Section of rabbit uterus incubated in substrate containing UDPG and then stained with Gram's solution. Black color is absent. ×50.

FIG. 5. Section of rat uterus incubated in substrate containing glucose-1-phosphate and then stained with Lugol's solution. Black color in myometrium and luminal epithelium indicates phosphorylase activity. Rat was treated with estrogen. ×50.

FIG. 6. Section of rat uterus incubated in substrate containing UDPG and then stained with Gram's solution. Dark color is not present. Animal was treated with estrogen. ×50.

tongue and heart of the rabbit and rat a positive response for phosporylase was observed in the muscle cells. The same organs demonstrated a positive response in the muscle cells for UDPG-glycogen transferase (Fig. 1, 2).

Phosphorylase activity in the uterus of the rabbit was confined to the myometrium and to the walls of the blood vessels. The greatest activity was observed following estrogen treatment (Fig. 3). In the uteri of ovariectomized or ovariectomized-hormone treated rabbits it was not possible to identify UDPG-glycogen transferase activity (Fig. 4).

In the rat uterus phosphorylase activity was observed not only in the myometrium but also in the luminal epithelium and again the greatest activity was observed following estrogen stimulation (Fig. 5). As in the rabbit uterus, it was not possible to identify UDPG-glycogen transferase activity in the uteri of the ovariectomized or the ovariectomized-hormone treated rats (Fig. 6).

Discussion. These observations on phosphorylase activity in the rabbit uterus confirm the previous results on rabbits(4). On the other hand, unlike my previous observations in the rat uterus(5), enzymatic activity was found not only in the myometrium but also in the luminal epithelium. The reason for this difference in localization of the enzyme in the rat uterus is not apparent. Wistar rats were used in both experiments but the animals were purchased from different animal farms.

Following the technic of Takeuchi and Glenner(3) it was not possible to identify UDPG-glycogen transferase in the uterus of the ovariectomized or the ovariectomized-hormone treated rabbits and rats, although this enzyme was clearly demonstrated in sections of the tongue and heart incubated in the same medium and at the same time.

Recently Hess and Pearse(6) observed a difference in the activities of phosphorylase and UDPG-glycogen transferase in muscle fibers. In fibers which have a large diameter, phosphorylase activity was very strong whereas in fibers of the small (red) type, a high activity of UDPG-glycogen transferase was found. In muscle fibers of uniform diameter (tongue) little difference in the two activities was detectable.

It should be pointed out that while it was impossible to demonstrate glycogen synthesis following incubation with UDPG, it is possible that glycogen may have been syn-

thesized from UDPG but since phosphorylase is at a high concentration, the polysaccharide is broken down and cannot be identified. With this reservation, on the basis of the present observations, it may be suggested that in the uterus of the rabbit and rat, glycogen synthesis occurs from G-1-P by the action of phosphorylase and not through the UDPG pathway.

Summary. Phosphorylase activity was observed in the uterus of the rabbit and rat. In the rabbit uterus enzymatic activity was confined to the myometrium while in the rat phosphorylase activity was present in the luminal epithelium as well as the myometrium. In both animals enzymatic activity was highest following estrogen stimulation. In the uterine sections of the castrate and hormone treated animals it was not possible to demonstrate histochemically UDPG-glycogen transferase. The results seem to indicate that in the uterus of the rabbit and rat only one polysaccharide forming pathway is present and perhaps the polysaccharide is synthesized from G-1-P and not from UDPG through the enzyme UDPG-glycogen transferase.

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Release of Antidiuretic Hormone Due to Common Carotid Occlusion and Its Relation with Vagus Nerve.* (27741)

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In recent years there has been an increase in interest in the regulation of blood volume (1, 2, 3). Because of its action on water output through the kidneys, the antidiuretic hormone (ADH) has received particular attention. The homeostatic role of this hormone in blood volume regulation has been established by the finding that the circulating ADH concentration increases after the reduction of blood volume by hemorrhage(4). Although the posthemorrhage increase in circulating ADH concentration may partially be explained by a reduced rate of ADH inactivation(5), there is a definite and marked increase in rate of ADH release(6). In a series of experiments, Gauer, Henry and their co-workers(7) have demonstrated the role of left atrium volume receptors and vagus nerves in regulating the release of ADH. However, the extent to which other receptors in the vascular system can influence ADH release has not been systematically studied. In circulatory physiology, the occlusion of common carotid arteries is a commonly used maneuver to simulate systemic hypotension in eliciting sympathetico-adrenal activity. The possibility that such maneuver may also lead to ADH release was studied in the experiments reported here.

Methods. Healthy mongrel dogs apparently in normal hydration were used in these experiments. Food was withheld from the animals for about 18 hours prior to experiment. Free access to water was allowed. After the dogs had been anesthetized by intravenous injection of sodium pentobarbital (33 mg/kg), the common carotid arteries were exposed low in the neck. A femoral artery was cannulated for measurement of arterial pressure with the use of Sanborn pressure transducer and recorder. The degree of anesthesia was kept at a relatively constant level by intermittent injection of small quantities of sodium pentobarbital

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