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Effects of Some Steroids on Glycogen Metabolism in Uterus and Skeletal Muscle.* (23756)

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While injections of estrogen result in the deposition of glycogen in the rat uterus(1,2, 3), efforts to produce this response with other steroids such as testosterone, adrenal cortical extracts and progesterone have failed(3,4) with one exception(5). The present study concerns an investigation of (a) ability of certain steroids to elevate uterine glycogen in the spayed rat, (b) effects of combinations of these steroids with estrogen on glycogen deposition and (c) their effects on mobilization of glycogen after epinephrine treatment. Glycogen levels in a representative skeletal muscle were determined concomitantly.

Materials and methods. Virgin female rats weighing 180-220 g were spayed 6-9 days before hormones were administered. In all experiments, the steroids‡ (except estradiol) were injected subcutaneously in 2 mg doses daily for 3 days just prior to autopsy. Estradiol benzoate (either 0.5 or 50 μg doses) was injected subcutaneously 48 hours prior to autopsy. Epinephrine hydrochloride (Parke Davis, Co.) was administered intraperitoneally in doses of 50 μg per 100 g of body weight 1 hour before the rats were sacrificed. Primarily for the study on skeletal muscle glycogen, all rats were fasted 24 hours before autopsy. The rectus femoris muscle and the uterus were removed from anesthetized (Nembutal) rats, rapidly weighed on a torsion balance and digested immediately in hot KOH. The methods used for the digestion and precipitation of the glycogen in these tissues were those previously described(3,6). The anthrone procedure(7) was employed to determine the concentration of glycogen in the tissues and the latter is reported as mg of glucose per 100 g of tissue (wet weight). The data on the uterus are reported in terms of total uterine weight, although most of the glycogen present is in the myometrium(3).

Results. When groups of spayed rats were given injections of the several steroids listed in Table I, the uterine glycogen levels of rats receiving testosterone and desoxycorticosterone were slightly but significantly higher than those of the spayed controls. The inability to demonstrate this effect of testosterone previously(3) was probably due to inadequate hormonal treatment (0.5 mg per day for 2 days). Among the several steroids tested, only testosterone produced a considerable increase in uterine weight similar to that observed in rats after adequate estrogen treatment. Thus, it is possible that the deposition of glycogen in uterine tissue (particu-

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TABLE I. Effects of Steroids on Glycogen Content of Uterus and Skeletal Muscle of Spayed Rats.

Treatment	No. of animals	Uterine wt (mg)	Glycogen (mg/100 g) Uterus	Glycogen (mg/100 g) Leg muscle
None (spayed)	14	116 ± 6*	78 ± 3	364 ± 13
Testosterone	7	256 ± 25†	129 ± 7†	574 ± 19†
Desoxycorticosterone	8	116 ± 11	107 ± 7†	412 ± 11
Cortisone	6	114 ± 9	74 ± 3	458 ± 17†
Cortisol	8	64 ± 5	113 ± 19	519 ± 36†
11-Desoxycortisol	8	83 ± 6	69 ± 5	464 ± 35
Pregnenolone	5	94 ± 17	86 ± 5	426 ± 16

* Mean ± stand. error of mean. † Mean significantly greater than controls, $P < .01$.

larly in the myometrium) induced by testosterone and estrogen is intimately related to the mechanism by which these hormones exert their anabolic effects, as suggested by previous work(6). The slight elevation in uterine glycogen following injection of desoxycorticosterone cannot be explained similarly since this steroid failed to stimulate uterine growth. However, glycogen storage in the uterus is not necessarily a concomitant of uterine growth, for increases in uterine glycogen have been obtained with doses of relaxin which did not alter uterine weight(8). The failure of the uterus to increase in weight following treatment with the other steroids tested agrees with previous findings(9,10).

The rectus femoris muscles of rats injected with testosterone, cortisone and cortisol contained considerably more glycogen than those of control animals. Pregnenolone increased skeletal muscle glycogen slightly but not significantly at the P value chosen ($< .01$). Previously testosterone and cortisone were shown to increase the glycogen content of skeletal muscle(6,11,12), while cortisol was reported to lack this activity in the gastrocnemius muscle of the normal rat(13). Other than the fact that a different muscle was used, it is difficult to understand why the latter investigators failed to obtain a response with this steroid.

The next study was made to determine the effects of some of these steroids on the deposition of glycogen induced by estradiol. Relatively large amounts of cortisone or cortisol were injected together with a dose of estradiol (0.5 μg per rat) which significantly increased uterine glycogen without producing a maximum response. It was found that neither of these adrenal cortical steroids significantly affected the deposition of uterine glycogen resulting from the action of estradiol (Table II). Progesterone also fails to modify the effect of estradiol on glycogen deposition in the uterus(4).

These results warrant a reconsideration of the role played by estrogen in the stimulation of uterine glycogen synthesis. It is well known that uteri removed from rats previously treated with estrogen have high rates of aerobic and anaerobic glucose uptake, as measured by the rate of disappearance of glucose from the incubation medium(14-17). The *in vivo* accumulation of glycogen in the uterus after estrogen administration has been considered(15,16) as the direct result of accelerated uptake of glucose from the blood. If this opinion is valid, cortisol, which inhibits the estrogen-stimulated *in vitro* uptake of glucose by the uterus (17), might also impair the *in vivo* accumulation of uterine glycogen. In the present experiment, however, neither cortisol nor cortisone interfered with the deposition of uterine glycogen induced with estradiol. The possi-

TABLE II. Effect of Some Steroids on Deposition and Mobilization of Glycogen in Estradiol-Treated Spayed Rats.

Treatment	No. of animals	Glycogen (mg/100 g) Uterus	Glycogen (mg/100 g) Leg muscle
Estradiol (.5 μg)	7	216 ± 19*	400 ± 27
+ cortisone	9	175 ± 11	501 ± 31
+ cortisol	6	205 ± 9	586 ± 50†
Estradiol (50 μg)	20	321 ± 12	450 ± 14
+ cortisone	8	315 ± 14	479 ± 18
+ epinephrine	6	161 ± 13†	294 ± 19
+ cortisone & epinephrine	11	73 ± 12†	323 ± 27

* Mean ± stand. error of mean; effect of estradiol can be noted by comparing with spayed controls, Table I.

† Mean significantly different from estradiol-treated controls, $P < .01$.

bility exists that insufficient quantities of the cortical steroids were injected to successfully compete with the amount of estradiol administered, although sufficient amounts were present to stimulate the deposition of glycogen in the rectus femoris muscle (Table II). On the other hand, it is conceivable that interference with uterine glucose uptake (at the glucokinase level) does not seriously impair the deposition of glycogen under estrogen stimulation, since there is no evidence which indicates definitely that blood glucose is the material utilized in the synthesis of uterine glycogen.

An experiment was also performed to determine the influence of cortisone on the glycogenolytic action of epinephrine in the uterus. Spayed rats were given doses of estradiol which elevated uterine glycogen to near maximal levels and a dose of epinephrine was employed which lowered uterine glycogen significantly. Another group of similarly treated spayed rats were given cortisone in addition. The glycogenolytic action of epinephrine in the presence of cortison was increased markedly and still lower glycogen levels resulted (Table II). Thus, cortisone potentiated the glycogenolytic action of epinephrine in the uterus.

It was previously demonstrated that in skeletal muscle (rectus femoris) cortisone inhibited(12) and desoxycorticosterone potentiated(18) the glycogenolytic action of epinephrine. The glycogen levels of the rectus femoris muscle were also determined in the above experiments and the results indicated that epinephrine decreased the glycogen of the leg muscle to a similar extent in rats either with or without cortisone treatment (Table II). Thus, an inhibition of the glycogenolytic action of epinephrine was not exhibited. However, the dose of epinephrine employed was quite large and probably nullified the inhibiting effect of the cortisone treatment, since previous work(12) stressed the importance of the proper dose relationship in order to demonstrate this antagonism of epinephrine by cortisone.

Summary. (1) Among a series of steroids which were tested for ability to elevate the glycogen level of the uterus of the spayed rat, testosterone and to a very slight extent, desoxycorticosterone were effective. (2) Cortisol elevated skeletal muscle glycogen, contrary to previous findings. (3) Cortisone and cortisol *in vivo* did not interfere with the deposition of uterine glycogen induced by the injection of small doses of estradiol. (4) Cortisone enhanced the glycogenolytic action of epinephrine in the uterus.

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