estradiol, though cholangio-fibrosis, and cirrhosis of the liver was more marked in the latter groups. The increase in the weight in the liver of rats on low protein diet is proportionally much smaller than the decrease in the level of the amino acid oxidases, accordingly a possible dilution of the enzyme in the liver cannot be taken as an explanation. In previous experiments(7) both components of the D-amino acid oxidase, the co-ferment riboflavin and the protein was estimated separately in liver of rats on protein free diet. The decrease in the amino acid oxidase in the livers could not be accounted for by the decrease of the riboflavin in the livers, and was thought to be due to a decrease of the specific enzyme protein. The amino acid oxidases in other organs were not studied. In view of the active participation of the liver in the protein metabolism, the finding in the present experiment that on reduction of dietary proteins a decrease of the amino acid oxidase occurred in livers only, might be of physiological importance.

Summary. 1. D-amino acid oxidases in livers and kidneys of rats on a low protein diet with or without addition of alpha estradiol have been compared with those of rats on a full diet. 2. The levels of D-amino acid oxidases in the kidneys was the same in all 3 groups. 3. In livers of rats on low protein diet, irrespective of feeding of alpha estradiol, the level of D-amino acid oxidases dropped to about half of that found in rats on full diet.

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Histochemical Studies on Glycogen Deposition in Uterus of the Rat* III. Effect of Starvation. (20373)

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It has been shown recently that glycogen is deposited in the smooth muscle of the uterus of the rat under the influence of estrogen, whereas progesterone, androgen and adrenal cortical extract each fail to produce this effect (1-3). This action of estrogen on the uterus is independent of both the adrenal cortex and hypophysis(2,3), but is, in a sense, analogous to the role played by the hormones of the latter organs in the deposition of glycogen in the liver and skeletal muscle. The glycogen content of the liver and skeletal muscle remains within normal limits in adrenalectomized(4) and hypophysectomized(5) animals provided a good nutritional state is maintained. Relatively short periods of fasting, however, result in a sharp reduction of liver glycogen in adrenalectomized animals and of both liver and skeletal muscle glycogen in hypophysectomized animals, although normal levels are promptly restored following treatment with the appropriate adrenal cortical or hypophyseal hormones(6,7).

The glycogen content of the uterus of intact and estrogen-treated castrate rats is not reduced by fasting(3). Whether estrogen can induce glycogenesis in the uterus after depletion of carbohydrate reserves, however, is not

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TABLE I. Amount and Distribution of Glycogen in Uteri and Livers of Fasted and Unfasted Rats Treated with Estrogen.

Treatment*	No. of animals	Uterus: Myometrium: Outer long.	Uterus: Myometrium: Inner circ.	Uterus: Endometrium: Epithelium	Uterus: Endometrium: Glands	Uterus: Endometrium: Stroma	Liver
Not fasted							
Ovariectomized	5	++++	++	+	0	0	++++
Ovariectomized-hypophysectomized	6	++++	++	++	0	0	+++
Fasted							
Ovariectomized	5	++++	++	+	0	0	+
Ovariectomized-hypophysectomized	7	++++	++	+	0	0	0

* All animals given one subcut. inj. of 20 μg of estradiol dipropionate and sacrificed 48 hr later.

known. The present experiments were designed, therefore, to determine the efficacy of estrogen in inducing glycogen deposition in the uteri of ovariectomized and ovariectomized-hypophysectomized rats starved prior to the beginning of hormonal treatment.

Materials and methods. Twenty-three adult female Wistar rats were bilaterally ovariectomized and divided into 4 groups as follows: 1) 5 animals were injected with 20 μg of estradiol dipropionate‡ at 3 P.M. of the 10th day after ovariectomy and were sacrificed 48 hours later; 2) 5 animals were treated as in Group 1 except all food was withheld beginning at 9 A.M. of the day on which they were injected with estrogen; 3) 6 animals were hypophysectomized on the 5th day after ovariectomy, injected with 20 μg of estradiol dipropionate at 3 P.M. on the 10th day after hypophysectomy and were sacrificed 48 hours later; 4) 7 animals were treated as in Group 3 except all food was withheld after 9 A.M. of the day on which they were injected with estrogen. When not fasted, the rats were maintained on Purina Chow *ad libitum*. Drinking water was available at all times. Crystalline hormone was injected subcutaneously, the dose being dissolved in 0.20 ml of cottonseed oil. At the termination of the experiment the animals were killed with chloroform and were autopsied immediately. They were examined grossly to ascertain the complete removal of the glands in question. Small pieces of uterus and liver were fixed in cold picro-alcohol-formalin and glycogen was subsequently demonstrated by the periodic acid-leucofuchsin technic previously described(1).

Observations. The results are summarized in Table I. In both the ovariectomized and ovariectomized-hypophysectomized rats fed *ad libitum* the deposition of glycogen in the uterus was similar to that described in our previous studies, *i.e.*, considerable amounts accumulated in the longitudinal layer of the myometrium and in the mesometrial region of the circular layer, smaller amounts being found in the remainder of the circular muscle. No glycogen was detected in either the epithelium of the endometrial glands or in the cells of the stroma.

In our previous studies glycogen deposition was not observed in the lining epithelium of the uterine lumen. However, in the present experiments, due presumably to the considerably larger dose of estrogen used, glycogen appeared in the lumenal epithelium. It was somewhat variable in amount and distribution in each uterus, some areas of the epithelium being free of the material, others containing small to moderate quantities. The over-all deposition was somewhat greater in the hypophysectomized castrates than in the castrates with intact hypophyses, but probably no special significance should be ascribed to this observation.

The livers of the fed ovariectomized rats showed extensive glycogen deposition, the parenchymal cells throughout the hepatic

‡ Estradiol dipropionate used in these studies supplied through the courtesy of the Ciba Pharmaceutical Products, Inc., Summit, N. J.

lobules being more or less uniformly filled with granules. In the fed hypophysectomized castrates, however, the amount of gylcogen in the cells in the outer one-third of the lobules was considerably reduced.

The glycogen content of the uteri of the starved ovariectomized and ovariectomized-hypophysectomized rats was not discernibly different from that of the fed ovariectomized animals described above. The liver glycogen in the starved animals, on the other hand, was greatly reduced compared with that present in the fed rats. In the starved ovariectomized animals there was sparse deposition only in the cells in the central portions of the lobules, whereas in the hypophysectomized castrates glycogen was completely absent.

Discussion. Carbohydrate metabolism is regulated to a considerable extent by the pancreas, anterior pituitary and adrenal cortex through their hormonal influence on the utilization of glucose by skeletal muscle and other peripheral tissues and through their effects on the storage and release of glucose by the liver. It should be noted that these various endocrine factors which affect carbohydrate metabolism in the living animal are not necessarily essential to the biochemical processes concerned in the storage and utilization of sugars, *e.g.*, in the hypophysectomized-adrenalectomized animal the levels of blood glucose and liver glycogen are maintained within normal ranges provided the animals are well nourished. Furthermore, many of the biochemical processes involved in carbohydrate metabolism have been demonstrated to proceed *in vitro* in the absence of all hormonal influence.

Little interest has been taken to date in the carbohydrate metabolism of smooth muscle or in its regulation by the endocrine glands. The observations of Walaas(3) and of the present authors(1,2) on the glycogenetic effect of estrogen on the uterine muscle in the rat have indicated that the synthesis and deposition of glycogen in this tissue proceed in the absence of the pituitary and adrenal cortex; the present study has demonstrated that estrogen remains effective as a stimulant for glycogenesis even if the carbohydrate stores of the animal are first depleted by starvation. The biochemical mechanisms involved remain obscure. It seems reasonable to assume, however, that the effect of estrogen is directly on the uterine muscle either through facilitation of glycogenesis from blood glucose, despite the lowered concentration of the latter, or through promotion of glyconeogenesis.

The deposition of glycogen in the uterine epithelium of the animals in the present study runs counter to our earlier experience in the intact cyclic rat and in the castrate treated with smaller amounts of estrogen. The present dosage of 20 μg of estradiol dipropionate is double or more that previously used and was selected in hope that a larger amount of glycogen would be deposited in the uterine muscle, thus facilitating microscopic study. Since we have re-examined our earlier material and have found no more than traces of glycogen deposited in the uterine epithelium of the intact cyclic rats, the present epithelial response may be considered to be "non-physiologic".

Summary. Relatively short periods of fasting result in the rapid disappearance of glycogen from the liver and skeletal muscle of hypophysectomized animals. The present experiments have demonstrated that, in spite of the depletion of carbohydrate reserves in the starved hypophysectomized castrate rat, estrogen promotes the deposition of glycogen in the uterine musculature in amounts comparable to those deposited in well-nourished animals. In this respect this action of estrogen on the uterus is analogous to that of certain of the hormones of the anterior hypophysis and adrenal cortex on the deposition of glycogen in the liver and skeletal muscle.

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