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A Histochemical Study on Some Dehydrogenases Concerning Carbohydrate Metabolism in the Uterus of Castrated Rat under Treatment with Ovarian Hormone

Jutaro TAKAHASHI, Tadahiko HOSHINO and Saburo TAKEUCHI

Department of Animal Husbandry, Faculty of Agriculture, Tohoku University, Sendai, Japan

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Summary

A histochemical study of the relationship between the changes of glycogen, the four enzymes concerning carbohydrate metabolism and ovarian hormone was made at various points during the progestational stage induced experimentally in the rat uterus.

The strong glycogen deposition is shown in the muscle, especially in the longitudinal layer at 12 hr after a single dose of 1 μg estrone administration.

The four enzymes (SDH, LDH, NADDH and G6PDH) have indicated a higher activity in the epithelium, subepithelial layer and uterine gland at various times from 6 to 24 hr after a single injection of estrone.

Activation of these dehydrogenases which are concerned with carbohydrate metabolism by estrone seems to have an important role in ovo-implantation and decidual reaction in the progestational uterus.

There are many reports concerning the relationship between dehydrogenases in carbohydrate metabolism and ovarian hormone in the rat and mouse uterus (1–9). However, little is yet known about the timecourse changes of dehydrogenases related to carbohydrate metabolism during the progestational stage in the rat uterus. Accordingly, in the present study, a histochemical observation of the relationship between the changes of glycogen, the four enzymes concerned with carbohydrate metabolism and ovarian hormone was made at various points during the progestational stage induced experimentally in the rat uterus.

Materials and Methods

Mature virgin female rats of the Wistar strain, weighing 180–280 g, were housed in an air-conditioned room (22±1°C) under regular illumination conditions (light on at 7:00 a.m., off at 19:00 p.m.). Vaginal smears of all animals were

recorded prior and throughout the experiment. Pseudopregnancy was induced by faradic stimulation of the cervix at proestrus and estrus. As shown in Fig. 1, the estrus period on which the vaginal smear shows the cornification was noted as day 1 of pseudopregnancy. The animals were divided into four groups. Each subgroup consisted of four or five rats. Ovariectomy was performed on all animals on day 2 of pseudopregnancy and followed by daily injections of 4 mg of progesterone for 9 days. In order to observe the effects of estrone, the animals of groups 1 and 3 were injected with a single dose of 1 μg estrone at the morning of day 8 of pseudopregnancy. These hormones were administered subcutaneously in 0.2 ml of sesame oil.

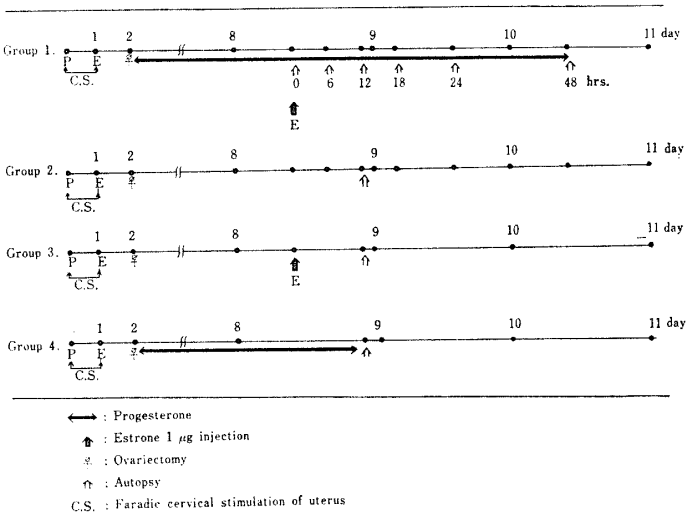

FIG. 1. Experimental design for ovariectomy and treatment of ovarian hormone.

In group 1, animals were killed at 0, 6, 12, 18, 24 and 48 hr after estrone administration. In groups 2, 3 and 4, the animals were killed at 10:00 p.m. on day 8 which is the corresponding time to 12 hr after estrone injection on day 8 of pseudopregnancy in group 1.

PAS positive substance and four dehydrogenases concerned with carbohydrate metabolism i.e. Succinate dehydrogenase (SDH), Lactate dehydrogenase (LDH), NADH dehydrogenase (NADDH) and Glucose-6-phosphate dehydrogenase (G6PDH) in the rat uteri were investigated by the method of the previous report (9).

PAS positive substance and enzyme activities were observed under the light microscope and indicated in the grade + to ### for each layer of the uterus.

Results

PAS positive substances and glycogen; The change of PAS positive substances obtained here is shown in Fig. 2. The glycogen deposition, disappearing by the pretreatment of salivary test, occurs in the muscle layer at 18–24 hr after estrone

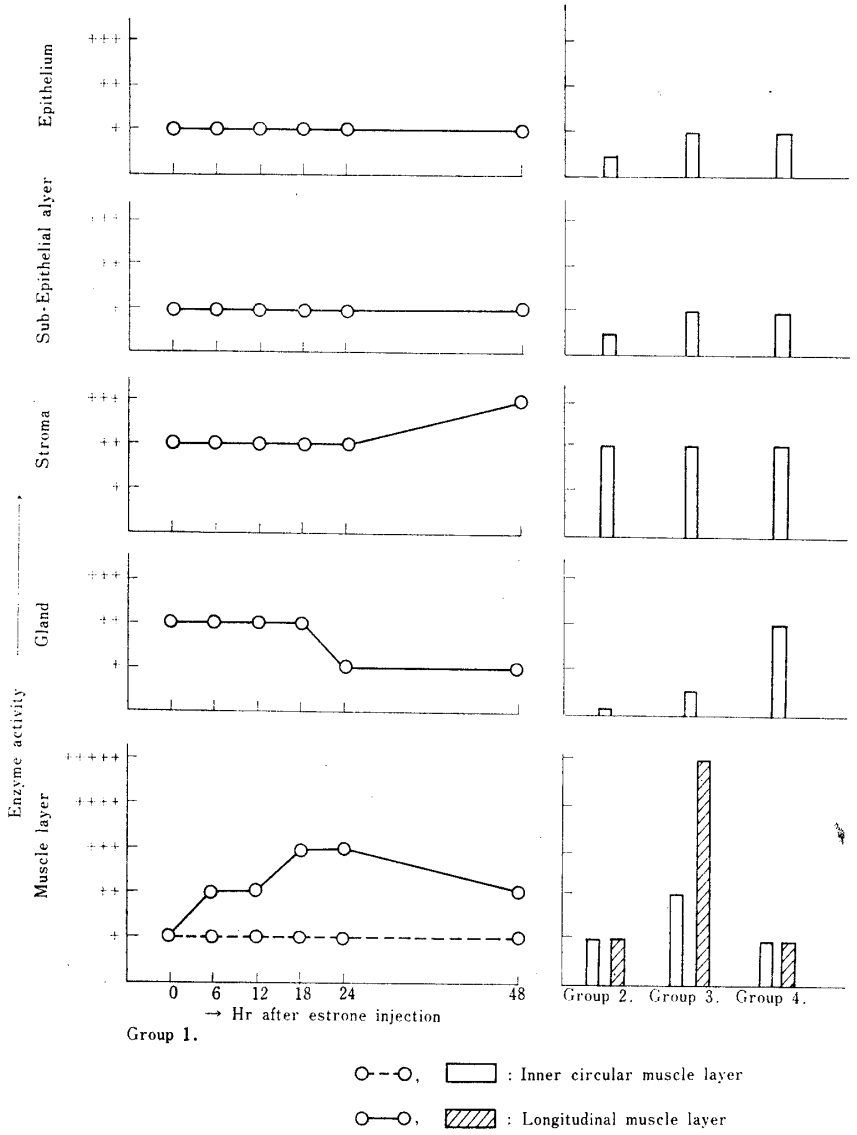

FIG. 2. The changes of PAS positive substance in the uteri of each group.

administration, but not in the epithelium, subepithelial layer, stroma and uterine gland. In group 3, strong glycogen deposition is shown in the muscle layer, especially in the longitudinal layer at 12 hr after estrone administration.

Dehydrogenases; The changes of dehydrogenase activity in various stages after estrone treatment are shown in Fig. 3–6. The change of SDH activity is shown in the epithelial layer, subepithelial layer and uterine gland, in which SDH activity tends to increase at 12–24 hr after estrone injection, while it is not evident in the stroma and muscle layers of group 1. In group 4, the activity is high in the epithelium and uterine gland compared to groups 2 and 3 (Fig. 3). LDH is active in the epithelium and subepithelial layer at 12 hr after estrone treatment. In groups 3 and 4, the activity is high in the epithelium in comparison with group 2 (Fig. 4). As shown in Fig. 5, the change of NADDH activity is only observed in

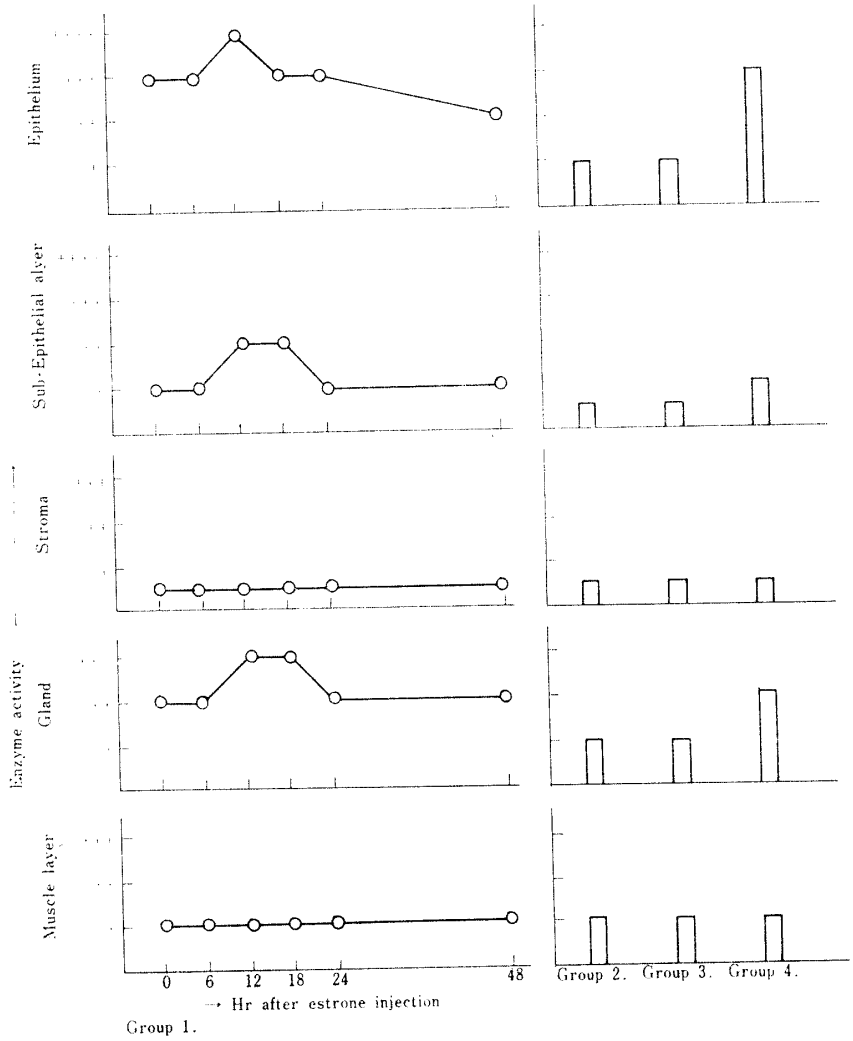

FIG. 3. SDH activity in the uteri of each group.

the epithelium. The change of G6PDH was also observed in the epithelium and was apt to be active at 6, 12 and 18 hr after estrone administration as shown in Fig. 6. The activity of G6PDH in group 4 is higher than that in groups 2 and 3 in the epithelium.

Discussion

PAS positive substances and glycogen

The deposition of glycogen occurs in the longitudinal muscle layer of the uterus and tends to increase at 6–12 hr and be maximum at 18–24 hr after estrone administration. In group 3, strong glycogen deposition is observed in the longitudinal layer at 12 hr after estrone administration. Gregoire et al (10) reported that the relation between the dose of estrogen and the amount of glycogen deposited is not linear nor does respond all portions of the uterus in a similar manner; while the progesterone loweres the glycogen content in all portions of the uterus. Further-

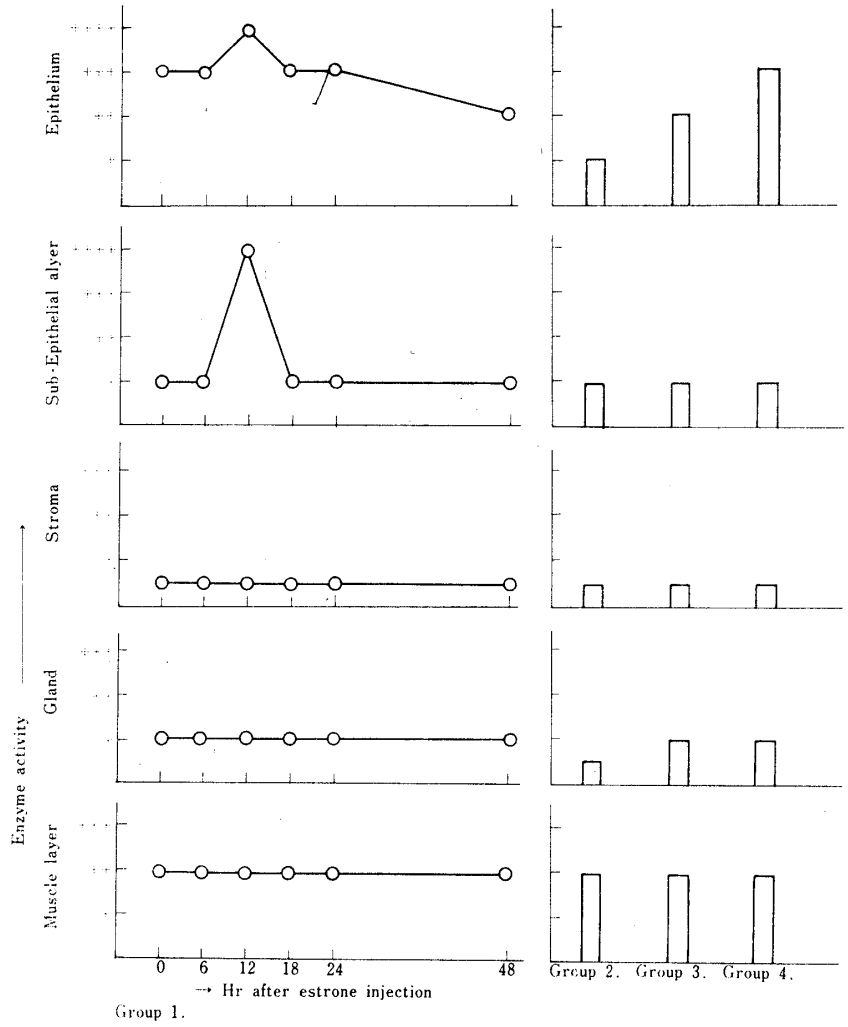

FIG. 4. LDH activity in the uteri of each group.

more, Rosenbaum and Goolsby (5) have reported that estradiol and estradiol benzoate are able to cause glycogen deposition in the myometrium and the stromal cells distributed about the endometrial glands and beneath the luminal epithelium. The results obtained here are on the whole in agreement with these reports indicating that glycogen deposition is estrogen-dependent in the rat uterus. There was a visible change and comparatively a high level of glycogen deposition on the 3rd and 4th days of intact pseudopregnancy in a previous study (9), but, in this experiment, these was no change or low level in any layer except for the muscle layer of the uterus. These results might be due to the difference of hormonal conditions between normal pseudopregnancy and experimentally induced pseudopregnancy.

Dehydrogenases

The relation between these oxidative enzymes in the uterus and ovarian

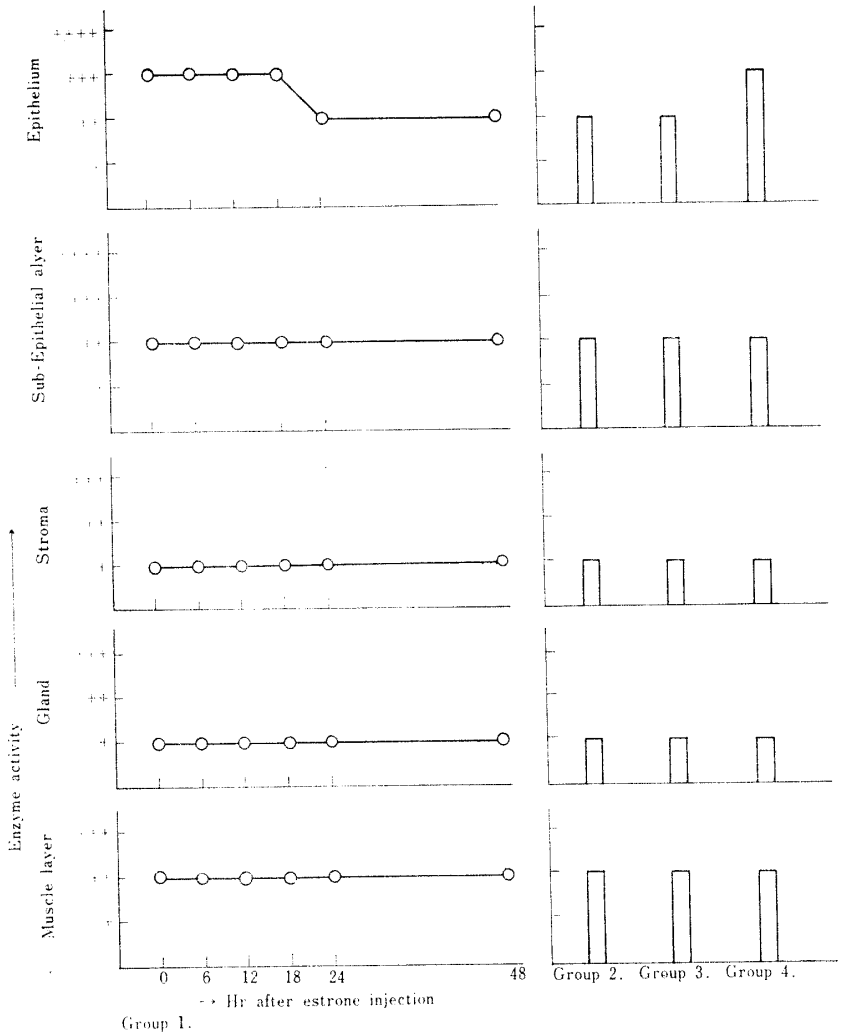

FIG. 5. NADDH activity in the uteri of each group.

hormone has been discussed by many previous workers. It has been reported that the SDH activity may be initiated by estrogen in the rat and mouse uterus (2, 4, 6, 7), but may be progesterone-dependent in the endometrium of rabbit (11) and ewe (12). In this experiment, when castrated animals receive progestrone administration and are then injected with a single dose of 1 μg estrone subcutaneously on day 8 of pseudopregnancy, the SDH shows a high activity in the epithelium, subepithelial layer and uterine gland at 12–18 hr after estrone injection. The castrated animals treated with only estrone (group 3) shows a low activity of SDH in every portion of uterus, while the animals under progesterone treatment (group 4) show a higher activity in the epithelium. Bever et al (1) have reported that the administration of estradiol-17β, estrone and estriol significantly affect the specific activity of the lactic dehydrogenase-DPNH oxidase system in the uteri of ovariectomized rats. In this study, administration of estrone results in a slight activation of LDH in the castrated rat with no hormoen treatment (group

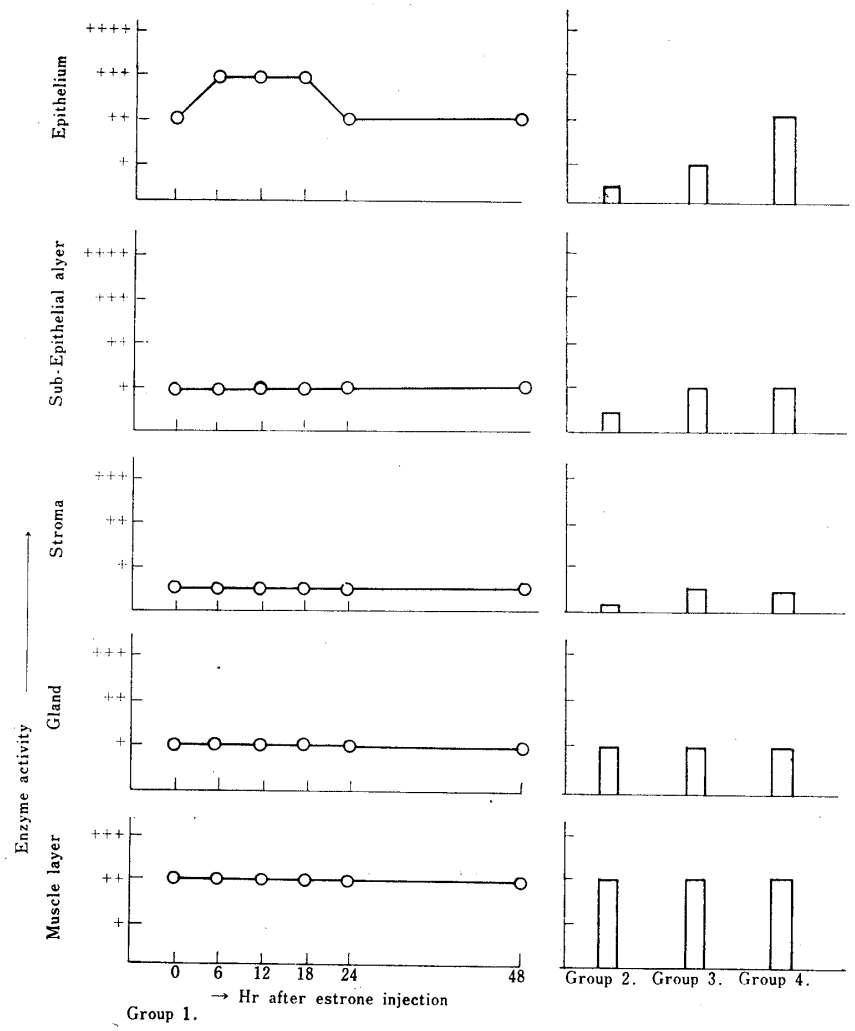

FIG. 6. G6PDH activity in the uteri of each group.

3), while animals of group 4 showed a higher activity in the epithelium. In group 1, the LDH activity was maximum at 12 hr after estrone administration in the epithelium and subepithelial layer, which is almost in agreement with the report of Bever et al (1). It has been reported that the activity of G6PDH in the uterus decreases after castration and increases after estradiol administration in the rat (2). In the present experiments, the G6PDH activity is low in all portions of the uteri in group 2. With a single dose of estrone, G6PDH is apt to show slight activity in the epithelium (group 3), while the castrated rats given progesterone treatment (group 4) show comparatively a high G6PDH activity in the epithelium and subepithelial layer. In group 1, G6PDH shows a high activity at 6, 12 and 18 hr after estrone administration.

These enzymes of the progestational uterus of rats, are inclined to be activated at 6—18 hr after estrone administration in the epithelium, subepithelial layer and uterine gland. Considering these results, it is thought that these dehydrogenases

are controlled by the balance between estrogen and progesterone. When the animals are ovariectomized, given daily treatments of 4 mg progesterone and then injected with a single dose of 1 μg estrone on the 8th day of pregnancy, the blastocysts grow rapidly at 12–24 hr and implantation occurs at 30 hr after estrone administration (13). Furthermore, when a single dose of estrogen was given at 12 to 24 hr before traumatization, a successful decidual reaction can be induced in the ovariectomized rats which were receiving 4 mg of progesterone daily (14).

The period of influence of estrogen on the development of blastocysts and on uterine sensitivity for decidual reaction generally corresponds with that the time of activation of dehydrogenases in the uterus. On the 4th day of intact pseudopregnancy, these dehydrogenases indicated a higher activity in the epithelium, subepithelial layer and uterine gland (9). The activity of these enzymes has increased in the uterus at this time, suggesting the carbohydrate metabolism i.e. Emden-Meyerhof system, TCA cycle and Hexose monophosphate shunt are activated by estrogen in the progestational uterus. The activation of these dehydrogenases concerned with carbohydrate metabolism seems to have an important role in ovo-implantation and decidual reaction in the progestational uterus of rat.

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Explanation of Plate 1~3

Plate 1

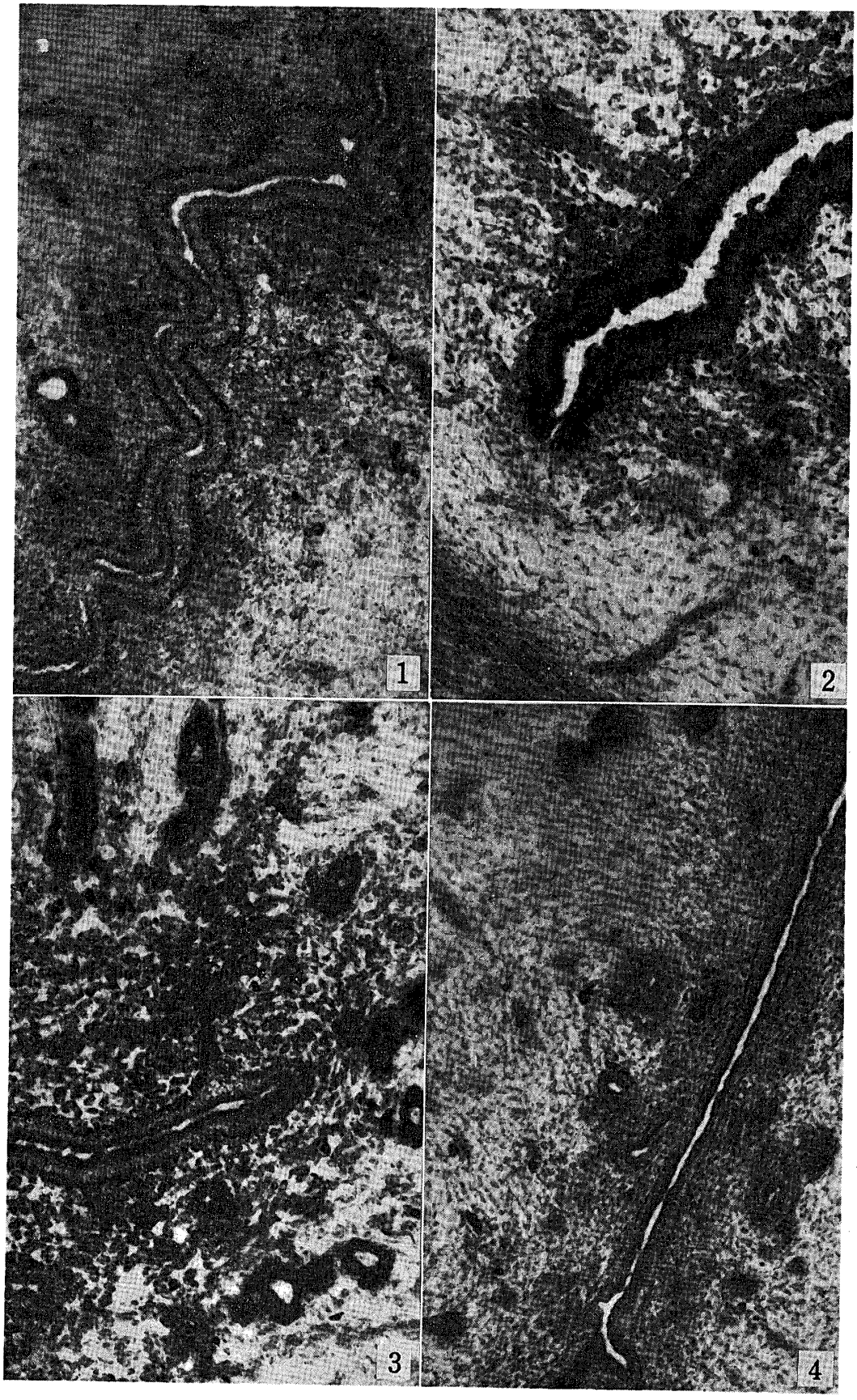
Explanation of Plate

1. SDH activity at the time of estrone administration (0 hr) in the uteri of group 1. ×100

2. SDH activity at 12 hr after estrone administration in the uteri of group 1. The strong activity is shown in the epithelium. ×100

3. SDH activity at 18 hr after estrone administration in the uteri of group 1. The activity decreases slightly in the epithelium in comparison with figure at 12 hr. ×100

4. SDH activity at 48 hr after estrone administration in the uteri of group 1. SDH is low active in the epithelium, subepithelial layer and uterine glands. ×100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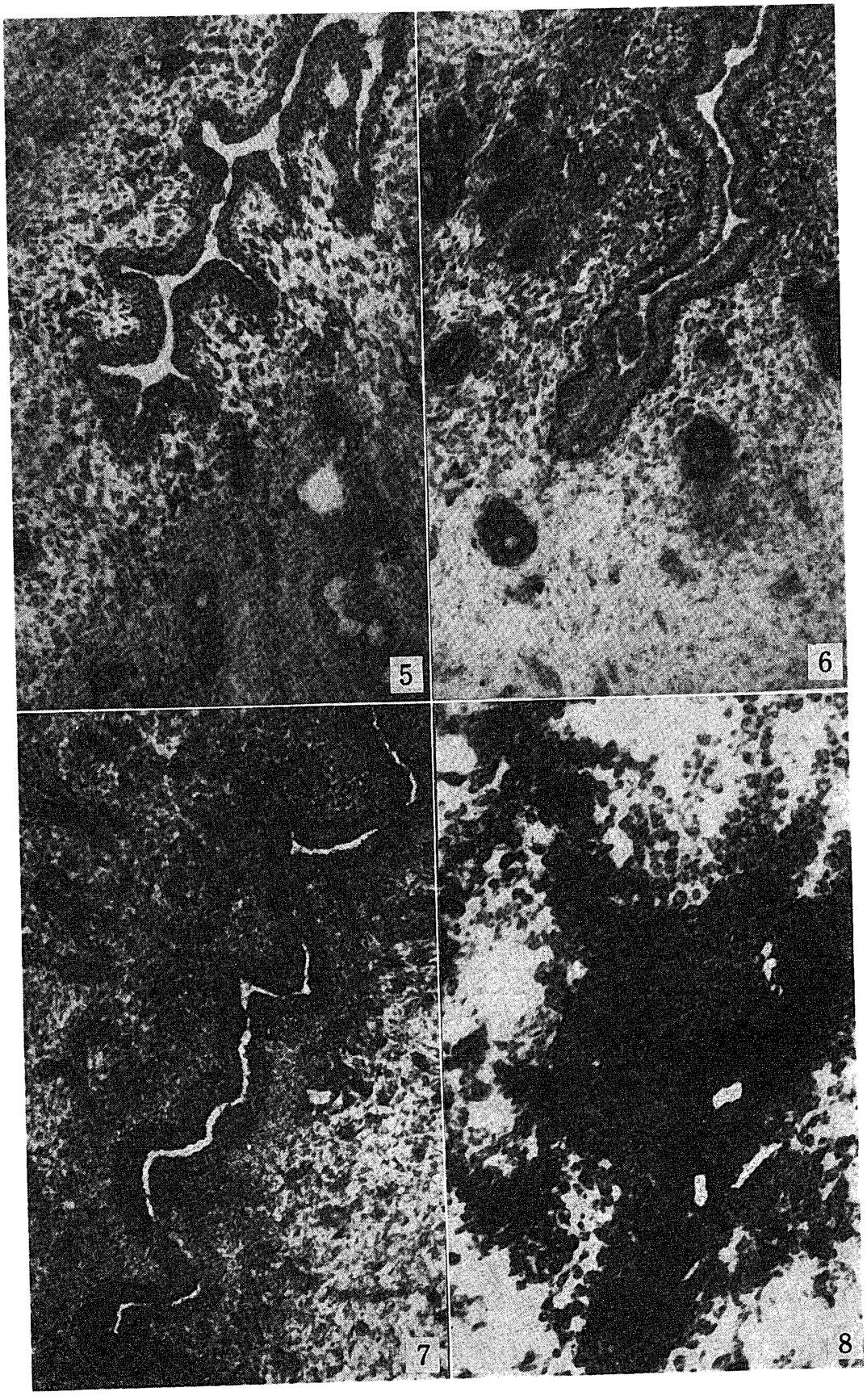
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Plate 2

Explanation of Plate

5. SDH activity in the castrated and no hormone treatment rat (group 2). The activity is low in every layer of the uterus ×100

6. SDH activity in the castrated and progesterone treatment rat (group 4). SDH is active in the epithelium and uterine glands. ×100

7. LDH activity at the time of estrone administration (0 hr) in the uteri of group 1. ×100

8. LDH activity at 12 hr after estrone administration in the uteri of group 1. Strong activity is shown in the subepithelial layer. ×100

Plate 3

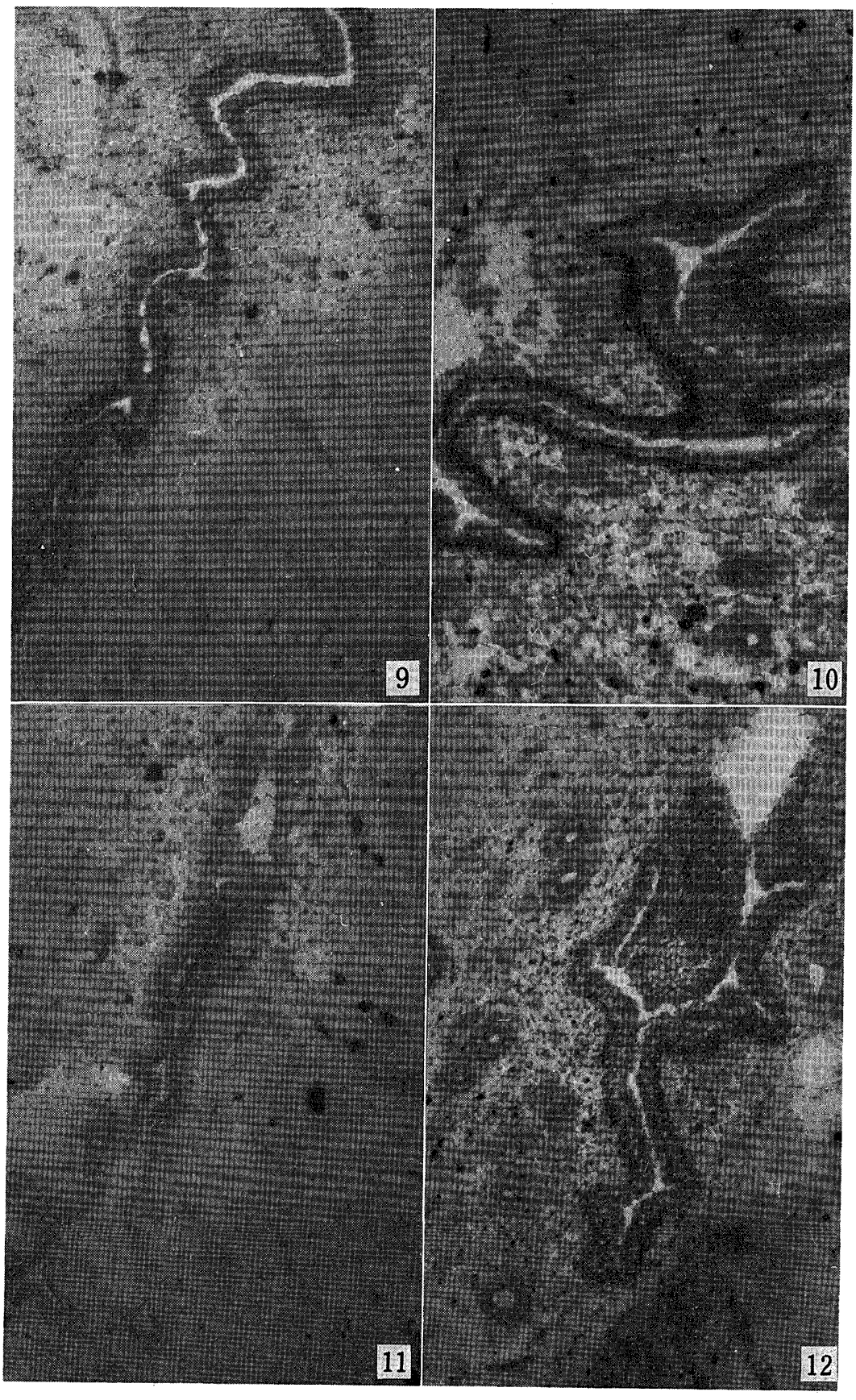
Explanation of Plate

9. G6PDH activity at the time of estrone administration in the uterus of group 1. ×100

10. G6PDH activity at 12 hr after estrone administration in the uteri of group 1. The activity is high in the epithelium. ×100

11. G6PDH activity in the uteri of group 2. The activity is low in the every layer of the uterus. ×100

12. G6PDH activity in the uteri of group 4. SDH is active in the epithelium. ×100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