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Glycogen Deposition in Squamous Metaplastic Epithelium of the Rat Uterus.* (22756)

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The deposition of glycogen in the normal uterus of the rat has been studied by both chemical(1) and histochemical methods(2). It has been demonstrated by chemical analysis that the total glycogen content of the rat uterus varies with the stage of the estrous cycle, being maximal during proestrus and minimal during diestrus. Histochemically, it has recently been shown that the glycogen of the rat uterus is limited to the muscle cells of the myometrium; in the proestrous animal the material is abundant throughout the longitudinal layer but is irregularly distributed in the circular layer while in the diestrous uterus, the glycogen content of the longitudinal muscle layer is greatly reduced and that of the circular muscle layer has disappeared entirely.

The distribution of glycogen in abnormal uteri, particularly in epithelial metaplasia of the rat uterus has not been reported. One of the methods used to produce and study uterine metaplasia in the intact rat is by placing the animal on a vit. A deficient diet(3). Using this method to produce epithelial metaplasia, the present investigation was undertaken to study, by the periodic acid-leucofuchsin technic, the distribution of glycogen in the metaplastic uterine glands of the intact rat.

Materials and methods. Twenty-five rats of the Wistar strain were used in these experiments. The animals were 20 to 22 days of age and weighed approximately 35 to 40 g when placed on the vit. A free diet.† The rats were killed with chloroform during the 9th to 14th week of the deficiency and the uteri were fixed immediately. A portion of each uterus

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† Vitamin A Test Diet U.S.P., Nutritional Biochemicals Corp., Cleveland, O.

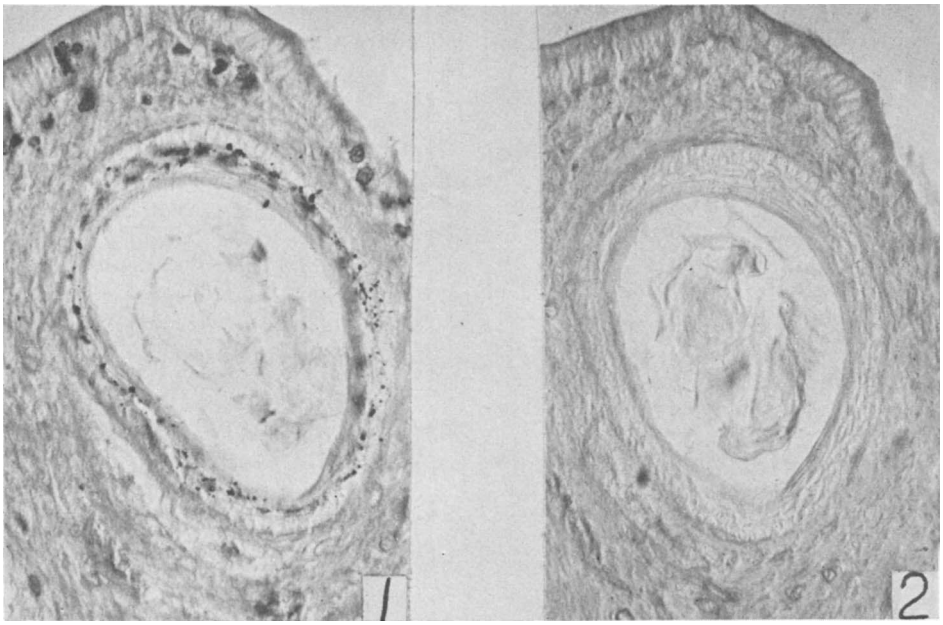

FIG. 1. Photomicrograph of a metaplastic gland following vit. A deficiency. The section was stained by the periodic acid-leucofuchsin method. Note dark glycogen granules in epithelium of metaplastic gland. × 410.

FIG. 2. Photomicrograph of a control section, close to that illustrated in Fig. 1, which was incubated in malt diastase to remove glycogen before staining. Note absence of glycogen granules from epithelium of metaplastic gland. Residual color in the section is due to staining of mucopolysaccharides and glycoprotein. × 410.

was fixed in cold modified PAF (10% formalin in 80% ethyl alcohol saturated with picric acid), cold acetone and in 10% formalin containing 2% calcium acetate. The present report is concerned with the study of glycogen deposition in epithelial metaplasia of the uterus following vit. A deficiency. After fixation in PAF the tissue was dehydrated, embedded in paraffin and sections were cut 12 μ in thickness. These were treated with periodic acid and then stained with leucofuchsin (4). Both glycogen and mucopolysaccharides are stained a reddish purple. In order to distinguish between them, control slides were incubated for an hour in 1% malt diastase buffered at pH 6.8 to remove the glycogen before applying the periodic acid-leucofuchsin procedure. No counterstain was used.

Observations. In the metaplastic glands that were observed in the uteri of the vit. A deficient animals, PAS positive material was present in the cells of the stratified epithelium. The granules were present primarily in the intermediate layer of the epithelium. In the control sections that were treated with 1% malt diastase, the material was not present indicating the presence of glycogen (Fig. 1 and 2).

The secretory product of the luminal and glandular epithelium that did not show any metaplastic changes was PAS positive but was resistant to 1% malt diastase which indicated that the material was not glycogen but a mucopolysaccharide.

The amount of glycogen observed in the tunica muscularis was variable; none was observed in the majority of the animals. In some of the animals only a trace of glycogen was observed in the longitudinal layer. The distribution of the glycogen in the uterine muscle was similar to that previously reported in the castrate and diestrous rat(2).

Discussion. The normal epithelium of the rat uterus, as was reported previously(2), does not contain any glycogen. However, the present results show that in the metaplastic epithelium following vit. A deficiency, glycogen granules are present in the cells of the

new epithelium.

The significance of glycogen deposition in squamous metaplasia of the uterus following vit. A deficiency and the biochemical enzyme systems involved is not known at the present time. There is evidence that the most important single factor which determines the rate of mitosis in tissue, such as mammalian epidermis, is the energy supply within the cells which depends upon the intracellular availability of glucose derivatives(5). If it can be assumed that because of increased mitotic activity the energy requirements of metaplastic epithelial cells is greater than for normal uterine epithelial cells, then the glycogen present in the metaplastic cells may be a source of readily utilizable energy.

The present results on glycogen deposition in epithelial metaplasia differs from the observation made in the human(6). In one instance a single squamous metaplastic cervical gland of one patient was reported to be free of glycogen. Since there appears to be a difference in glycogen deposition in squamous metaplasia, investigations are under way to determine whether the deposition of glycogen in stratified squamous metaplasia is peculiar to the changes following vit. A deficiency or whether the deposition of glycogen occurs in all epithelial metaplasia.

Summary. In the epithelial metaplasia of the rat uterus that occurred following vit. A deficiency, glycogen was observed in the stratified epithelium. An earlier report showed that the normal uterine epithelium of the rat uterus contains no glycogen.

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Effect of Methotrexate Therapy upon Choriocarcinoma and Chorioadenoma. (22757)

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Folic acid is known to be essential for the growth of the female genital tract and for normal embryonic development. Hertz *et al.* demonstrated the inhibition of estrogen-induced growth in the chick oviduct and monkey uterus in animals maintained on a folic acid deficient diet or treated with antagonists of either folic acid or adenine(1,2,3). Reduction of a previously high titre of gonadotropin occurred following a short course of methotrexate in a hypophysectomized woman with metastatic melanoma.*

The present report includes observations on the effect of a repeatedly administered and intensive regimen of methotrexate upon the urinary excretion of chorionic gonadotrophin and upon the clinical course in 2 patients with choriocarcinoma and one patient with chorioadenoma destruens.

Methods. The diagnosis was established by histological evidence. The initial extent of the disease was determined by physical examination and by radiological survey. Other observations included recording on each day of (a) fluid intake and output, (b) caloric intake, (c) the hemogram, and (d) the body weight. Each week x-rays were taken and measurement of palpable or visible lesions was recorded. Urinary gonadotropin determinations were carried out by a modification of the method of Klinefelter *et al.*(4). Gonadotropin values are expressed as "mouse uterine units" per 24 hours. The titres on

* Unpublished.