that use of this method will facilitate the more accurate choice of chemotherapeutic agents in treatment of individual tumors even of the same morphologic class, and perhaps be an effective means of rapidly screening metabolic antagonists as to their effect upon individual tumors.

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Effect of Beta-Carotene and Cortisone on Vaginal Epithelium of Vitamin A Deficient Rats.* (24214)

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Conversion of beta-carotene to Vit. A is partially influenced by tocopherols, Vit. B_{12}, antibiotics, phosphates, insulin and thyroxin (1). Recently Clark and Colburn(2), utilizing biochemical analyses, found that administration of cortisone to rats on stock laboratory diet or Vit. A free diet caused rapid reduction in quantity of Vit. A in liver and kidney. They further demonstrated that only when large amounts of beta-carotene were administered to cortisone-treated rats was there adequate conversion of beta-carotene to Vit. A and deposition of Vit. A in liver and kidney. They suggested that conversion of beta-carotene to Vit. A is impaired in cortisone-treated rats.

The results obtained by Clark and Colburn (2) seem to indicate that cortisone-treated animals would eventually become Vit. A deficient and exhibit manifestations of the deficiency. However, it is also possible that blocking of the conversion of beta-carotene to Vit. A by cortisone may result in quantitative reduction of the Vit. A stored in body depots, and may not produce pathological changes in organs sensitive to Vit. A deficiency. With this question in mind, and by utilizing cornification of the vaginal epithelium of the rat as criterion of Vit. A deficiency(3), the present investigation was undertaken to determine whether beta-carotene in the presence of cortisone treatment would cause cornification of vaginal epithelium of Vit. A deficient rats to disappear.

Methods. Fifty-one rats of Wistar strain were placed on a Vit. A free diet‡ on day 20 of life. Daily weighings and vaginal smears were begun 3 weeks after initial feeding of Vit. A free diet.

When the Vit. A deficient animals exhibited vaginal cornification continuously for 3 to 4 days, they were subjected to the following procedure. In Group I (17 animals), the rats received daily either 6, 12, 24, 48, or 480 μg of beta-carotene§ orally for 10 days. Rats of Group II (6 animals) received 2.5 mg of cortisone acetate subcutaneously/day for 10 days but received no beta-carotene. Animals in Group III (19 animals) for 10 days received daily subcutaneous injections of 2.5 mg of cortisone acetate and in addition either 12, 24, 48, or 480 μg of beta-carotene orally. Rats in Group IV (9 animals) received 2.5 mg of cortisone acetate subcutaneously/day for 3 days prior to dual treatment of cortisone and beta-carotene daily for the next 10 days. Dosages of beta-carotene used for animals of Group IV were 12, 24, or 48 μg.

At the end of prescribed treatment, the animals were sacrificed and the vagina of each animal removed and fixed in Zenker's solution. After fixation the tissue was dehydrated, imbedded in paraffin, and sectioned at

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‡ Vit. A Test Diet U.S.P., Nutritional Biochemicals Corp., Cleveland, O.

§ The beta-carotene was supplied through the courtesy of Hoffmann-LaRoche, Nutley, N. J.

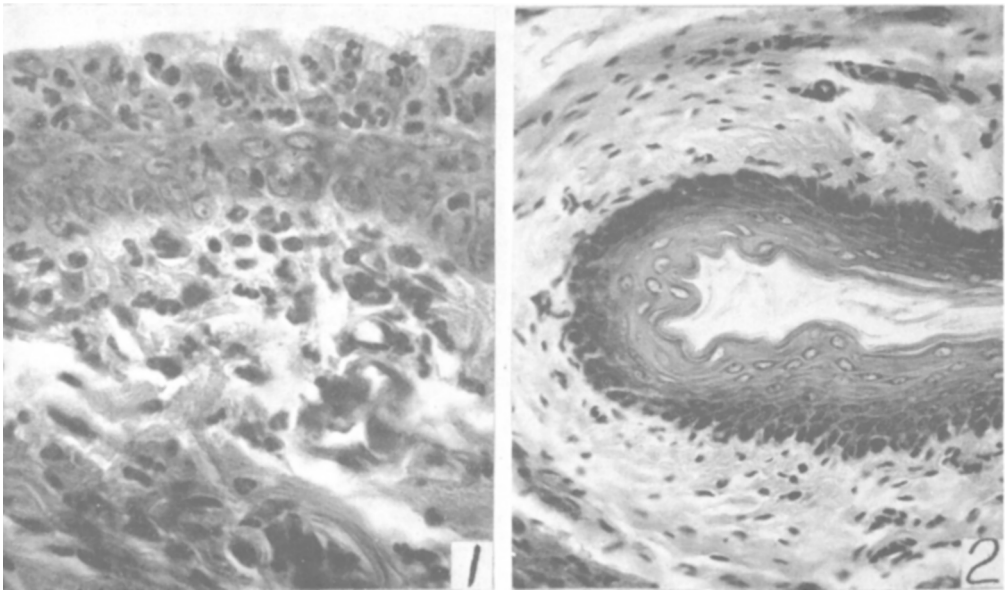

FIG. 1. Photomicrograph of portion of vagina of vit. A deficient animal treated with beta-carotene. Cornified epithelium of vit. A deficiency is replaced by columnar and cuboidal shaped cells. Neutrophiles are also present throughout. Sections of vagina of vit. A deficient rats treated with beta-carotene and cortisone are similar to this photomicrograph. Weigert's hematoxylin and eosin (×390).

FIG. 2. Photomicrograph of portion of vagina of vit. A deficient animal treated with cortisone. Vaginal epithelium is stratified squamous keratinized and like that of vit. A deficient animals. Weigert's hematoxylin and eosin (×230).

8 μ and stained with Weigert's hematoxylin and eosin.

Results. The first manifestation of Vit. A deficiency was abnormal cornification of the vagina. This appeared from 4 to 5 weeks after beginning of the feeding of Vit. A free diet. Beginning with the fifth week the animals steadily decreased in weight. Since cornification of the vagina and decrease in body weight are well known symptoms of Vit. A deficiency, further consideration of those changes will not be presented.

After the third day of treatment with beta-carotene alone (Group I), vaginal smears of all animals contained only nucleated cells and neutrophiles. The keratin layer of the stratified epithelium of the vagina was replaced by columnar and cuboidal shaped cells (Fig. 1). The new cells at surface of stratified epithelium had large nuclei and contained many intracytoplasmic vacuoles. Also, neutrophiles were present among cells of the stratified epithelium and in the lumina of the vaginae. The results demonstrate that beta-carotene suppresses abnormal cornification of the vagina in Vit. A deficient rats.

In animals that received 2.5 mg cortisone acetate alone (Group II), abnormal cornified epithelium of the vagina continued unchanged (Fig. 2). This demonstrated that cortisone acetate alone had no effect on cornified epithelium of Vit. A deficient rats. The changes observed in vaginal epithelium of rats which received the hormone and beta-carotene concurrently (Group III), were similar to those observed in the animals that received beta-carotene alone, thereby demonstrating that beta-carotene does influence the epithelium of the vagina in the presence of cortisone. The alterations observed in the vaginae of animals in Group IV, receiving cortisone alone for 3 days prior to combined treatment of beta-carotene and cortisone, were similar to those observed in rats that received beta-carotene alone or beta-carotene and cortisone.

Discussion. The cuboidal and columnar shaped cells that replaced the keratin layer of the stratified epithelium of the vagina in

beta-carotene treated animals are similar to those which occur in the vagina of the rat after topical application of Vit. A(4,5). Our results, following beta-carotene treatment are similar to those of Sherwood, Brend and Roper(6) who observed nucleated cells in vaginal smears of rats following oral treatment with beta-carotene.

Since rats receiving cortisone alone gave no indication of alterations in the vaginal smear, it seems clear that changes observed in the beta - carotene or beta - carotene - cortisone treated animals must have been the result of conversion of beta-carotene to Vit. A.

No estrogenic activity follows local or subcutaneous treatment with cortisone(7,8), therefore, persistence of cornified epithelium in the vagina of deficient animals treated with cortisone was due to Vit. A deficiency, and not to treatment with the hormone. Under our conditions, therefore, cortisone had no demonstrable effect on conversion of beta-carotene to Vit. A.

The present observations do not completely contradict the work of Clark and Colburn(2), who found a reduction in the quantity of Vit. A stored in liver and kidneys. The possibility of cortisone having an effect on storing of Vit. A, and not on conversion of beta-carotene to Vit. A, must be recognized.

Summary. Abnormally cornified epithelium of the vagina induced by Vit. A deficiency disappeared when beta-carotene alone or beta-carotene and cortisone was administered. Cortisone alone did not modify the cornified epithelium of the vagina. The results demonstrate, therefore, that cortisone has no demonstrable effect on conversion of beta-carotene to Vit. A.

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Failure of Blood Glucose Levels to Reflect Hepatic Glycogenolysis; Experiences with Glucagon.* (24215)

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It is common practice in some experimental situations to measure hepatic glycogenolysis indirectly through changes in blood glucose levels. Accurate blood glucose determinations can be performed rapidly and blood samples can be obtained conveniently at any desired interval. Direct determination of liver glycogen, on the other hand, requires liver biopsy or sacrifice of the experimental animal, and it is not practicable to perform repeated determinations on the same subject during an acute experiment. On the basis of serial blood sugar determinations, it has been stated that glucagon is inactive when administered subcutaneously(1,2). In the course of our studies of glycogen metabolism, we have in several instances drawn erroneous conclusions from such indirect evidence. It is the purpose of this communication to call attention to the hazards of such indirect "measurement" of glycogenolysis and to emphasize the necessity of performing glycogen analyses.

Methods. Normal and adrenodemedullated male Sprague-Dawley rats, weighing 200 to 260 g, were used. Blood glucose was

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