Relation of Vitamin A Deficiency and Estrogen to Induction of Keratinizing Metaplasia in the Uterus of the Rat

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IT IS WELL known that a deficiency of vitamin A and also overstimulation with estrogen causes a keratinizing metaplasia of the epithelium of the rat uterus. Wolbach and Howe[1] were the first to carry out detailed studies of the morphologic changes produced by the withdrawl of vitamin A from experimental animals. They observed that stratified squamous keratinizing metaplasia developed in all types of epithelia through the following sequence of histologic changes: atrophy of the original epithelium, proliferation of the basal cells which displaced the original epithelium and finally a differentiation into a stratified squamous keratinizing epithelium. In the rat the uterine changes were first observed to occur in the endometrial glands. Metaplasia of the epithelium of the uterus of the guinea pig is more marked than for the rat.[2] In vitamin A-deficient mice metaplasia of the uterine epithelium has not been observed to occur.[3]

Selye, Thompson, and Collip[4] were the first among many investigators[5-14] to observe that prolonged treatment with estrogen produces metaplasia of the epithelium of the uterus. Although all authors agree that metaplasia of the uterine epithelium can be induced in experimental animals by overstimulation with estrogen, there is no agreement about the manner of origin and development of the abnormal epithelium.

From the data available in the literature on keratinizing metaplasia of the epithelium of the rat uterus, it is not clear whether avitaminosis A exerts its influence directly on the epithelium independent of estrogen stimulation or whether it is dependent on estrogen for the induction of the keratinizing metaplasia. The experiments to be described in this paper were designed to provide information on the manner of origin and development of stratified squamous epithelium in the uterus of the rat by overstimulation with estrogen and to provide information on the relationship of avitaminosis A to estrogen in producing keratinizing metaplasia of the epithelium of the rat uterus.

MATERIALS AND METHODS

Three separate experiments were carried out to study the problems mentioned above. Rats of the Wistar strain were used in all the experiments. The animals were killed with ether and the uteri fixed for 12 to 24 hours in Zenker's or Helly's solution. Serial sections were made of each uterus and every tenth section was mounted and stained with Weigert's hematoxylin and eosin.

Specific information on the execution of the experiments along with the observations made will be presented under appropriate headings below.

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OBSERVATIONS

I. The Manner of Development of Squamous Metaplasia Induced by Estrogen[15]

Starting on the seventh day of age the rats were treated weekly with a single subcutaneous injection of 2 mg estradiol dipropionate.* Animals were autopsied every three days from the 3rd to the 27th day after receiving the first injection. Others were killed on the 33rd and the 39th day after initial injection with estrogen.

Uterine metaplasia was first evident in the animals autopsied on the 18th day after the first injection of estrogen, (Table I). Epithelial metaplasia was observed to begin as multiple foci which were located along the horns of the uterus, (Fig. 1a). The abnormal epithelium was formed by proliferation of the luminal epithelial cells. The foci of epithelial metaplasia grow by continual division, undermine the original epithelium and eventually separate the original epithelium from the underlying connective tissue. In advanced conditions keratinized epithelium was present, (Fig. 1b). Destruction of the luminal epithelium was not observed to precede the formation of squamous metaplasia.

TABLE I

Induction of Epithelial Metaplasia in the Rat Uterus by Weekly Administration of 2.0 mg Estradiol Dipropionate Beginning at Seven Days of Age

Number of animals	Days after 1st injection autopsied	Number of animals showing metaplasia	Extent of squamous metaplasia
25	3–15	0	–
5	18	4	+
5	21	4	+++
5	24	4	+++
5	27	4	++
5	33	2	+++
5	39	4	++

II. Influence of Ovariectomy on Vitamin A-Deficiency Induction of Epithelial Metaplasia[16]

Rats 20 to 22 days of age were divided into four groups. In group I the animals were kept intact and placed on a vitamin A-deficient diet.* The rates in group II were treated as those in group I except that they received a dietary supplement of vitamin A. The animals in group III were bilaterally ovariectomized and placed on a vitamin A-deficient diet. In group IV the rats were treated as in group III except that they received a dietary supplement of vitamin A. Animals were autopsied from the 8th to the 13th week of the experiment.

In order to determine whether the daily food intake of the vitamin-deficient animals was an important factor in producing symptoms of avitaminosis A rats of Group III and Group IV were pair-fed.

Metaplasia of the uterine epithelium of the vitamin A-deficient intact rats was similar to that previously reported,[1] (Figs. 2c, d, e). However, no metaplasia of the uterus was observed in the vitamin A-deficient rats following ovariectomy (Table II; Fig. 2).

TABLE II

Influence of Ovariectomy on Vitamin A Deficiency Induction of Epithelial Metaplasia in the Rat Uterus

Group	Number of animals	Intact or overiecto-mized	Vitamin A	Presence of squamous metaplasia
I	30	Intact	Deficient	Yes
II	10	Intact	Supplied	No
III	30	Ovariecto-mized	Deficient	No
IV	10	Ovariecto-mized	Supplied	No

The results indicate that the ovaries also have an important role in producing metaplasia in the uteri of vitamin A-deficient rats. Of the ovarian hormones, it is probably estrogen that is concerned with the metaplastic changes that occur in the uterine epithelium, since progesterone is primarily concerned with secretory activity of the epithelial cells whereas estrogen is primarily concerned with stimulating mitosis and cell growth.

Since the rats of group III in the pair-fed series showed external manifestations of vita-

* The estradiol dipropionate used in this study was supplied through the courtesy of the Ciba Pharmaceutical Products, Inc., Summit, New Jersey.

* Vitamin A Test Diet U.S.P. XIV, General Biochemicals, Inc., Chagrin Falls, Ohio.

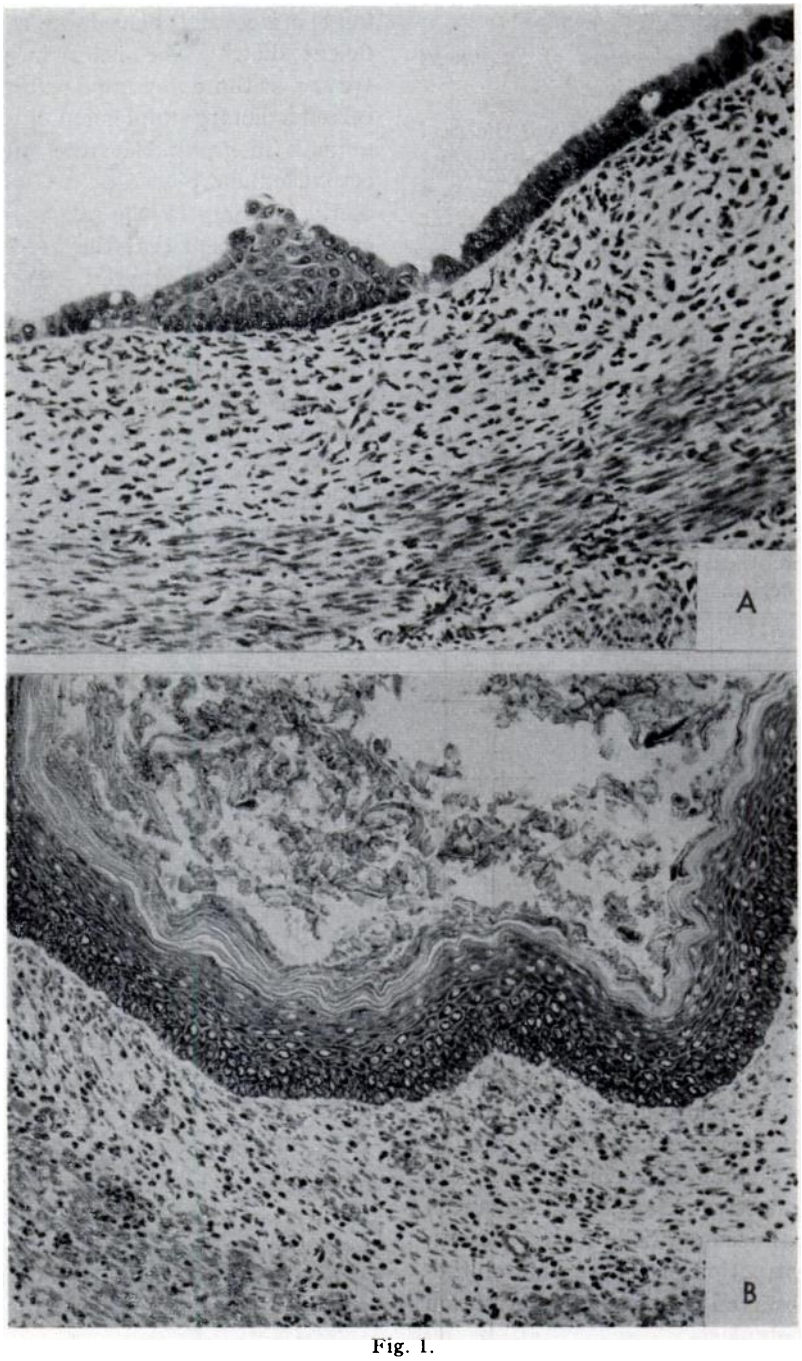

Fig. 1.

(a) Photomicrograph of a portion of the uterus showing the start of squamous keratinizing metaplasia following estrogen stimulation (hematoxylin and eosin) × 160. (b) Photomicrograph of a portion of the uterus showing extensive stratified squamous keratinizing epithelium following estrogen stimulation (giemsa stain) × 160.

min A deficiency and metaplasia of the lining epithelium of the trachea while the animals of group IV on the supplemented diet, which were pair-fed, exhibited no manifestations of vitamin A deficiency, it could be concluded that the changes observed were due to avitaminosis A and not to indirect effects of the deficiency.

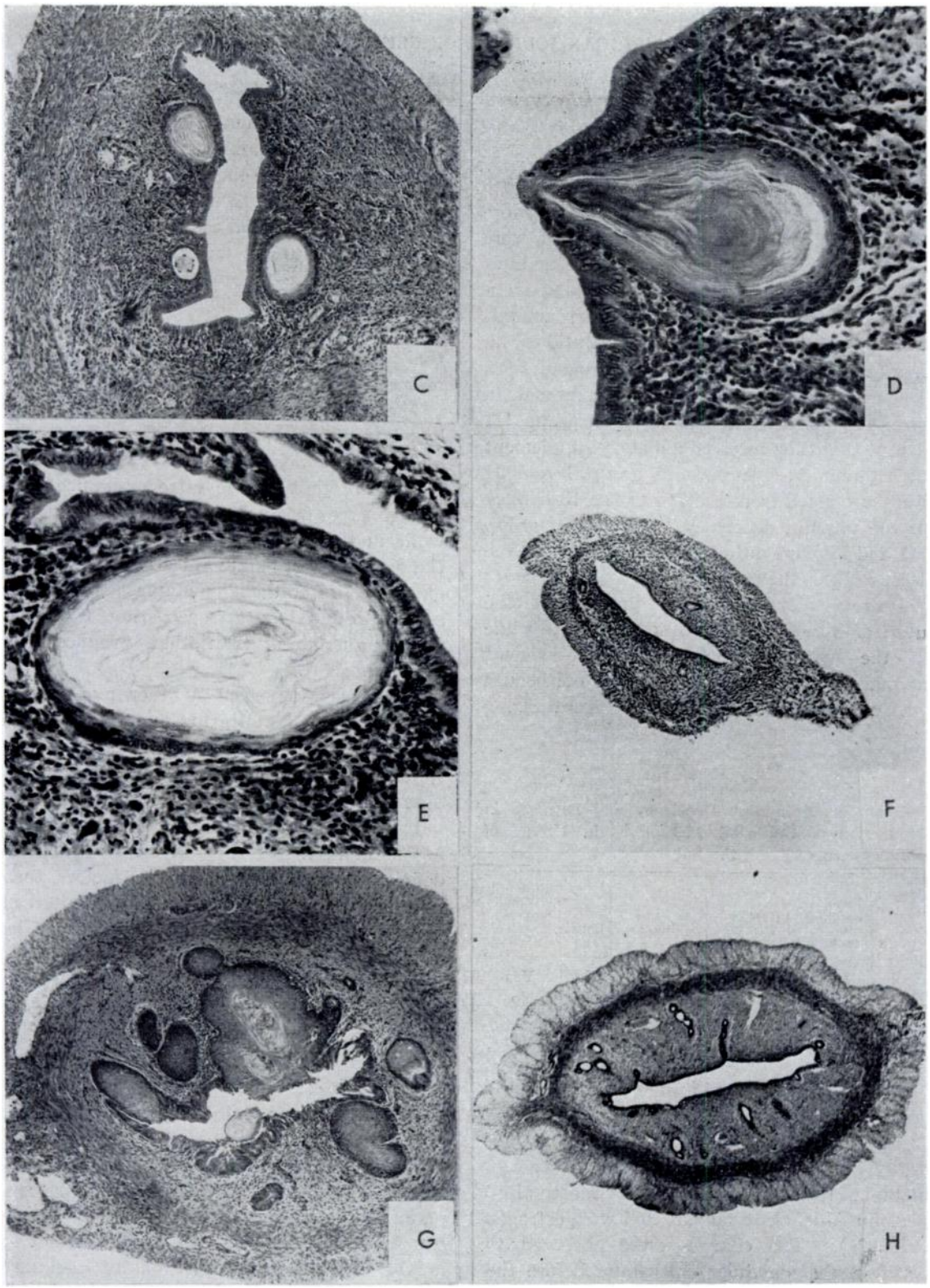

Fig. 2.

(c) Photomicrograph of a portion of the uterus of an intact vitamin A-deficient rat demonstrating stratification and keratinization of the glandular epithelium. The animal was autopsied during the 12th week of the deficiency (hematoxylin and eosin) × 70. (d) and (e). Photomicrograph of a portion of the endometrium of an intact vitamin A-deficient rat demonstrating extensive keratinization of the glandular epithelium. The animal was autopsied during the 12th week of the deficiency (hematoxylin and eosin) × 175. (f) Photomicrograph of the uterus of an ovariectomized vitamin A-deficient rat. No evidence of uterine metaplasia is present. The animal was autopsied during the 13th week of the deficiency (hematoxylin and eosin) × 55. (g) Photomicrograph of the uterus of an ovariectomized vitamin A-deficient animal treated with estrogen. Many foci of uterine metaplasia are demonstrated. The rat was autopsied during the 6th week of the experiment (hematoxylin and eosin) × 45. (h) Photomicrograph of the uterus of an ovariectomized animal on an adequate diet and treated with estrogen. No evidence of uterine metaplasia The animal was autopsied during the 9th week of the experiment (hematoxylin and eosin) × 30.

III. Relation of Vitamin A Deficiency and Estrogen in Production of Epithelial Metaplasia[17]

Rats 20 to 22 days of age were divided into four groups. In group I the animals were kept intact while the rats in group II were bilaterally ovariectomized and all the animals were placed on the vitamin A-free diet mentioned heretofor. In group III the animals were bilaterally ovariectomized, maintained on a vitamin A-deficient diet and treated with 1 μg estrogen every third day. The animals in group IV were treated as those in group III except that they received a dietary supplement of vitamin A. Animals in group I and II were autopsied from the 7th to the 13th week of the vitamin deficiency while rats of group III and IV were autopsied from the 5th to the 10th week of the experiment.

Uterine metaplasia was present in the uteri of intact vitamin A-deficient rats while in the ovariectomized vitamin A-deficient animals metaplasia of the uterine epithelium was not observed (Table III). Keratinizing metaplasia occurred in the ovariectomized vitamin A-deficient rats treated with estrogen (Fig. 2g). The changes were observed to occur in the glandular epithelium before the lining epithelium. Uterine metaplasia was not present in the ovariectomized animals on an adequate diet and also treated with estrogen (Fig. 2h).

TABLE III

Relation of Vitamin A Deficiency and Estrogen in Production of Epithelial Metaplasia in Uterus of the Rat

Group	Number of animals	Intact or ovariectomized	Vitamin A	Estrogen	Presence of squamous metaplasia
I	15	Intact	Deficient	No	Yes
II	15	Ovariectomized	Deficient	No	No
III	30	Ovariectomized	Deficient	Yes	Yes
IV	30	Ovariectomized	Supplied	Yes	No

DISCUSSION

The observations made in the present study demonstrate that keratinizing metaplasia induced by estrogen begins as multiple foci which are located along the uterine horns. These foci of epithelial cells extend toward the lumen of the uterus and also laterally from point of origin to undermine the original luminal epithelium. The superficial cells of this new cell growth become keratinized. By this process the entire luminal epithelium is replaced by a keratinizing epithelium.

The origin of epithelial metaplasia produced by estrogen stimulation differs from the metaplasia which occurs in vitamin A deficiency in that in the estrogen-induced the change takes origin in the luminal epithelium wnile in that following nutritional deficiency the changes originate in the endometrail glands. The epithelial metaplasia in the rat uterus produced by estrogen stimulation was more pronounced than that produced by avitaminosis A.

It has been suggested by other investigators that squamous keratinizing metaplasia of the uterus results from a local vitamin A deficiency regardless of any other factors.[18] The results obtained from the present investigations do not support that conclusion. If all keratinizing metaplasia is due to a local vitamin A deficiency regardless of any other factors, then such changes would be present in the uteri of ovariectomized vitamin A-deficient rats. The observations made in the present studies demonstrate rather clearly that uterine metaplasia following avitaminosis A occurs only when the epithelial cells are under the influence of estrogen. The synergistic action of vitamin A deficiency and estrogen in producing uterine metaplasia is evident by the pronounced metaplastic changes observed in the ovariectomized rats on a vitamin A-deficient diet and treated with estrogen as compared to the absence of changes in the ovariectomized animals on a vitamin A free diet or in the ovariectomized rats on an adequate diet treated with estrogen.

No definite relationship exists between vitamin A deficiency and estrogen in producing abnormal cornification of the vagina. The

change occurs in the intact as well as in the ovariectomized vitamin A-deficient rats.[19–21] It has been reported that cornification of the vagina following estrogen treatment is altered by excess vitamin A.[22,23] Recently, tissue culture technics have been used to study this relationship between vitamin A and estrogen in producing keratinized epithelium. The vagina has primarily been used for this study. It has been demonstrated that estrogen can produce cornification of the mouse vaginal epithelium *in vitro*.[24] Also, *in vitro*, keratinization of the rat vagina was observed to take place in cultures that contained a standard medium.[25] The keratinization that occurred was probably due to a lack of available vitamin A. The uterus, up to the present time, has not been used for *in vitro* studies. However, if such studies were undertaken interesting observations would be made on this problem of the relationship between vitamin A deficiency and estrogen in producing keratinizing metaplasia in the uterus of the rat.

These observations indicate that the abnormal growth that occurs in the uterus and that which has been reported to occur in the vagina following avitaminosis A cannot be considered to be the same. Although the end result, a keratinized epithelium, is the same the cytological processes involved in producing the change are different in the two organs. In the vagina the abnormal growth is more of a hyperplastic change and, as mentioned above, occurs in the intact and ovariectomized rat while in the uterus the growth is a true metaplasia and occurs only in the intact animal. This difference in response of the uterus and vagina to vitamin A deficiency may result from the difference in the embryology, morphology and physiology of the two organs. The conclusions drawn from observations made on the vagina in avitaminosis A or between vitamin A and estrogen cannot be generalized to include the uterus.

The question which arises in regard to keratinized epithelium is which of the two causes, estrogen or vitamin A deficiency, is the primary factor in producing the change. On the basis of the present work it can be concluded that vitamin A deficiency *per se* cannot be considered to be the primary factor in inducing keratinizing metaplasia in the uterus of the rat. Estrogen seems to have an equal role in producing the change. On the other hand, from the experimental data that are available on the rat vagina, vitamin A deficiency seems to be the primary factor in producing abnormal cornification.

Although vitamin A supresses the cornification of the vagina induced by estrogen, it cannot be definitely concluded that vitamin A plays a general role in the prevention of all keratin formation. In the epidermis of the male rat, topically applied vitamin A dissolved in sesame oil did not interfere with the keratinization process.[26] Likewise, in the guinea pig, vitamin A dissolved in oleic acid, linoleic acid or alcohol did not inhibit the formation of the stratum corneum of the skin.[27] Also, vitamin A did not prevent the estrogen-induced hyperkeratosis of the nipple epidermis in the guinea pig.[26]

The mode of action of vitamin A in maintaining normal uterine epithelium is not clear. Since vitamin A has not been demonstrated by fluorescence microscopy in epithelia that are known to undergo keratinization in depleted animals, it has been suggested that vitamin A *per se* is not responsible for maintaining normal epithelia but a metabolite of vitamin A may be.[28] It seems that in order to maintain the integrity of the uterine epithelia a balance exists between vitamin A and estrogen and when this balance is disrupted a keratinizing metaplasia occurs.

SUMMARY

Metaplasia of the uterine epithelium of estrogen-treated rats begins as many independent centers that grow and coalesce to produce a keratinized stratified squamous epithelium which replaces the original uterine epithelium.

The origin of epithelial metaplasia produced by estrogen stimulation differs from the metaplasia which occurs in vitamin A deficiency in that in the former the change takes origin in the luminal epithelium and the latter in the endometrial glands. The epithelium produced by estrogen stimulation is thicker and more

heavily keratinized than that produced by vitamin A deficiency.

Keratinizing metaplasia in the uterus of the rat was observed to occur only in intact animals on a vitamin A-deficient diet or in the ovariectomized, vitamin A-deficient rats treated with estrogen. The results demonstrate that estrogen has an important role in producing keratinized epithelium in avitaminosis A and the change cannot be considered to be due to only a local vitamin A deficiency.

From the present investigation it can be concluded that vitamin A deficiency is not the primary factor in producing keratinizing metaplasia in the uterus of the rat since estrogen plays a role in producing the change.

The mode of action of vitamin A in maintaining normal uterine epithelium is not known, but it seems that a balance may exist between estrogen and vitamin A in order to maintain the integrity of the uterine epithelium and when this is disrupted keratinizing metaplasia occurs.

The abnormal cornification of the vagina that occurs in vitamin A deficiency of the rat was compared with the uterine changes and it was concluded that the two are not the same. In the vagina the change is more of a hyperplastic change while in the uterus it is a true metaplasia. Therefore, the conclusions drawn from the observations made on the vagina in avitaminosis A and between vitamin A and estrogen cannot be generalized to include the uterus.

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