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I hereby recommend that the thesis prepared under my supervision by Walter J. Bo

entitled Study of Metaplasia in the Uterus of the Rat

be accepted as fulfilling this part of the requirements for the degree of Doctor of Philosophy

Approved by:

William B. Atkinson

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STUDY OF METAPLASIA IN THE UTERUS OF THE RAT

A dissertation submitted to the

Graduate School of Arts and Sciences
of the University of Cincinnati

in partial fulfillment of the
requirements for the degree of

DOCTOR OF PHILOSOPHY

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by

Walter J. Bo

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ABSTRACT

Metaplastic changes due to vitamin A-deficiency and overstimulation with estrogen were studied in the uterus of the rat. The investigation was concerned with the following problems: (1) the role of the ovaries in producing uterine metaplasia in vitamin A-deficient rats; (2) whether uterine metaplasia which is induced by estrogen is a gradual process or a rapid one which takes place a short time after the beginning of treatment; (3) whether the age of the animal at the start of estrogen treatment has any effect on the production of metaplasia; and (4) a cytologic comparison of the metaplastic processes resulting from vitamin A-deficiency with those resulting from overstimulation with estrogen.

In the vitamin A-deficiency experiments, histological studies were made of the uteri in: (1) intact and ovariectomized animals raised from weaning (3 weeks of age) on a vitamin A-deficient diet; and (2) intact and ovariectomized rats on the same diet supplemented with vitamin A. The animals were autopsied from the 8th to 13th week of age. In the estrogen experiments, the experimental animals received a single subcutaneous injection of 2.0 mg. of estradiol dipropionate per week. Uteri were studied histologically in: (1) intact rats treated for the first time on the 7th or the 21st day of age; (2) ovariectomized rats treated for the first time on the 21st day of age; and (3) untreated

controls. The animals were autopsied from the 3rd to 39th day after the first injection.

The metaplastic changes observed in the uteri of vitamin A-deficient intact rats were similar to those reported by previous authors. These consisted of stratification of the luminal epithelium and keratinization of the glandular epithelium. No metaplastic changes were found, however, in the uteri of vitamin A-deficient rats which had been ovariectomized. This difference in response indicates that ovarian estrogen plays an important role in producing uterine metaplasia and that vitamin A-deficiency should not be considered the sole factor.

A marked difference in response to overstimulation with estrogen was observed between the animals treated for the first time on the 7th day of age and those treated on the 21st day. Twenty-two of 30 animals treated on the 7th day developed pronounced metaplastic changes from the 18th to the 39th day after the first injection. Only 2 of 45 animals treated for the first time on the 21st day of age, however, showed metaplastic changes in the uterus in this same time interval. It was further noted that estrogen-induced uterine metaplasia began as numerous independent foci and not as an extension of the stratified cervical epithelium as had been suggested by previous authors. The metaplastic changes did not take place within a short time after the first injection, usually requiring about three weeks, but once metaplasia began the

process was rapid and the entire uterine epithelium was replaced by stratified squamous epithelium within several days. Some investigators have claimed that the presence of an inflammatory response was an important factor in producing uterine metaplasia. Since the majority of the animals in this experiment showed little or no evidence of an inflammatory reaction accompanying uterine metaplasia, inflammation does not seem to play an important role in producing this lesion.

Comparison of the metaplastic changes in the uterus due to vitamin A-deficiency and those due to overstimulation with estrogen revealed that there is a considerable difference in cytologic appearance. In the vitamin A-deficient rats the luminal cells showing the first indication of metaplasia extended into the lamina propria, while in the estrogen-treated rats the metaplastic cells bulged into the lumen of the uterus. Keratinization of the lining epithelium of the uterus in the vitamin A-deficient rats was not observed; however, keratinization was observed in the epithelium of the uterine glands of these animals. The estrogen-treated animals exhibited opposite effects. The lining epithelium became keratinized but the glandular epithelium did not. It seems probable, therefore, that estrogen and vitamin A-deficiency produce uterine metaplasia by different mechanisms, the details of which remain obscure.

INTRODUCTION

The term growth, although frequently used by biologists, is difficult to define. Weiss (1949) proposed to define growth as ".....the multiplication of that part of the molecular population capable of further continued reproduction, irrespective of whether or not accompanied by cell division.....we might say that immigration of molecules into the cell space as such does not constitute growth, but their assimilation into complex systems after the model of pre-existing indigenous systems does."

Growth of an organ generally means an increase in number of cells. However, cells of an organ must also differentiate in order to carry out their particular function. The differentiation of a cell depends upon its environment as well as its genetic makeup. The more highly specialized a cell becomes the less is its power to reproduce.

To the morphologist, the processes of growth and differentiation may be classified into three categories: alteration in size of cells, alteration in number of cells and alteration in structure of cells. A decrease in size of cells is known as atrophy, while an increase in size of cells is called hypertrophy. An increase in the number of cells is called hyperplasia, while a change in an adult type of cell to another adult type is termed metaplasia.

The present problem is concerned with metaplasia. This

term was first used by Rudolf Virchow in 1854 to indicate a change in the character of a tissue. He introduced the doctrine of histological substitution. One tissue at a certain point in the body could be replaced by an analagous tissue, or what he called an histological equivalent. An example given was columnar epithelium acquiring a squamous appearance. Virchow believed metaplasia to be a direct transformation of one type of differentiated cell into another.

Adami and McCrae (1914) defined metaplasia as follows: "Metaplasia is the postnatal production of specialized tissue from cells which normally produce tissues of other orders, and is an adaptation on the part of the cells to altered environment.....Metaplasia is not direct, but can be brought about only by a preliminary reversion to a vegetative type of cell, or where mother cells are present, by the development of cells modified by environment." The above definition is the one most generally accepted.

The present work will deal especially with metaplasia in the uterine epithelium of the rat. The three common experimental conditions used to produce metaplasia are: chronic irritation, stimulation to rapid growth, and vitamin A-deficiency. The two methods chosen to produce uterine metaplasia in the present study were vitamin A-deficiency and rapid growth brought about by overstimulation with estrogen since these have been reported to cause metaplastic changes in the luminal and glandular epithelium of the uterus.

REVIEW OF LITERATURE

The review will be considered under three categories. The first part will be concerned with vitamin A-deficiency, particularly with the manifestations exhibited in the uterus and vagina; the second, with the effect on the uterus of overstimulation with estrogen; the third, with the relationship between vitamin A-deficiency and estrogen in producing metaplasia in the uterus.

A. Vitamin A-Deficiency

Various investigators such as Emmet and Allen (1920), Stephensen and Clark (1920), Cramer, Drew and Mottran (1921) studied the effect of vitamin A-deficient diets on rats, but it was not until 1922 that Mori called attention to the changes which occur in the lining epithelium of certain organs such as the larynx, trachea, and ducts of submaxillary, sublingual and parotid glands of rats which had been fed a diet deficient in fat soluble vitamins. This author also observed changes in the lacrimal glands of animals suffering from xerophthalmia. The most striking change was the shrinkage of the secretory cells of the acini, giving the appearance of a single mass of nuclei. Mori contended that a gland in this condition could not produce tears; therefore, xerophthalmia resulted. The epithelium of the principal ducts of

the salivary glands showed cornification and desquamation of the cells. Mori also observed similar changes in the lining epithelium of the larynx and the trachea. This new epithelium that formed contained keratohyaline granules.

In 1925, Wolbach and Howe made the first detailed study of the changes in the lining epithelium of various organs due to vitamin A-deficiency. Rats 45-60 days old were placed on the deficient diet. Control animals were placed on the same diet supplemented with butterfat. The typical gross manifestations of vitamin A-deficiency such as humped posture, rough coat, emaciation and encrusted eyelids were observed. The histological changes in lining epithelia were easiest to follow in the trachea. The appearance of keratinized epithelium was preceded by atrophy of the original pseudostratified columnar epithelium. The change began by numerous foci which spread rapidly and undermined the original epithelium. The foci which were destined to produce stratified squamous epithelium were recognized as clumps of dark staining cells. These cells at first formed a syncytium with numerous mitotic figures. This was followed by keratinization of the superficial cells. In the uterus the authors observed the earliest changes in the endometrial glands. They also observed a responsiveness of the stroma suggesting a role in the formation of new growths of epithelium. However, they finally concluded that replacement of epithelium arises from focal proliferation of cells arising from the

original epithelium and not by differentiation or change of preexisting cells. The authors also maintained that the cells, due to their activity, had taken on neoplastic properties.

Parkes and Drummond (1926) made some observations on the uteri of vitamin A-deficient rats. The animals were on a vitamin A-deficient diet from 2 to 6 months. The authors found that the general appearance of the uteri was fairly normal. However, in one uterus they observed the beginning of an epithelioma.

Since the diet used by Mori (1922) and Wolbach and Howe (1925) was deficient in vitamins C and D as well as A, Goldblatt and Benischek (1927) tried to determine whether a single vitamin deficiency (vitamin A) could induce metaplastic changes. At the start of the experiment the rats were 28 to 35 days old. The authors obtained similar results to those of Wolbach and Howe (1925) but did not report changes in the uterus.

Tyson and Smith (1929) studied tissue changes associated with vitamin A-deficiency in rats placed on the vitamin A-deficient diet at 21 to 24 days of age. The animals were examined: (1) after a short time on the diet, (2) after they developed marked symptoms of vitamin A-deficiency, and (3) after they received vitamin A which corrected the symptoms of the deficiency. The principal changes observed were squamous metaplasia of cuboidal and columnar epithelium in certain

organs of the body and hyperplasia of other epithelia. The metaplastic changes involved the sublingual and submaxillary glands and the epithelium of the renal pelvis, trachea and bronchi. The authors made no mention of the uterus.

Wolbach and Howe (1928) studied the effect of vitamin A-deficiency in the guinea pig. This was done in order to establish a specific pathologic process irrespective of species. The animals were placed on the vitamin A-deficient diet and maintained on it for 105 days. Lesions of the eyes did not occur in guinea pigs as they did in rats, indicating a species difference in this respect. In 5 of the 6 animals the uteri presented striking enlargements. The walls were thickened in 3 of the uteri due to increase in size of the mucosa, and the lumina were filled with a white, pasty material. Keratinized epithelium was found before pronounced atrophy of the organs concerned had occurred. From their work the authors concluded that in the guinea pig, as in the rat, the observations suggested that the growth of the replacing epithelium was rapid and was not governed by the regulatory mechanisms which control growth of normal epithelium.

Tiden and Miller (1930) observed keratinization of the reproductive tract in monkeys placed on a vitamin A-deficient diet at the age of one to one and a half years. The first sign of the deficiency was a loss of weight which appeared 3 months after the start of the experiment. As in the

guinea pig, eye lesions were not found.

Wolfe and Salter (1931) studied the effect of vitamin A-deficiency in the albino mouse. The studies were made on a total of 50 mice, male and female, which were placed on the deficient diet when they reached a weight of 9 to 12 grams. Appearance of external manifestations of vitamin A-deficiency varied from 90 to 120 days. Animals with most advanced symptoms showed external signs similar to the ones described by Wolbach and Howe (1925). The gross postmortem changes observed were distention of the renal pelvis, which was filled with desquamated cells, and thickening of the bladder wall. They also observed a disappearance of body fat and a reduction in size of the salivary glands. The epithelium of various body organs was replaced by keratinizing cells which usually began as small clumps beneath the normal cells. These foci grew by peripheral extension beneath the original epithelium. The most common finding was an orderly layer of keratinized cells which seemed to begin at one point and grow in all directions. These histological changes were observed in the respiratory tract, submaxillary gland, bladder, prostate and seminal vesicles. The authors did not observe any changes in the uterus.

Extensive observations have been made on the effects of vitamin A-deficiency on the vagina. The first observation on excessive cornification of the vaginal epithelium in vitamin A-deficient rats was made by Evans and Bishop (1922).

The animals were not on a completely deficient diet so they grew normally for several months and did not suffer from xerophthalmia. However, the estrous cycles were prolonged and ovulation failed to occur in 100 per cent of the animals. The authors maintained that continuous cornification of the vagina was a more sensitive test for vitamin A-deficiency than xerophthalmia.

In 1927, Macy, Outhouse, Lang and Graham confirmed Evans' and Bishop's (1922) work. More detail as to the age and length of time of the experiment is included in this work. The rats were 21 to 26 days old when placed on the vitamin A-deficient diet. The first indication of cornification of the vaginal epithelium appeared on the 23rd day of the experiment, xerophthalmia appeared on the 34th day. They likewise concluded that the presence of cornified cells in the vagina was a more sensitive test of vitamin A-deficiency than xerophthalmia.

By 1930 three biological methods were used in quantitative determination of vitamin A: the growth method, the ophthalmic method, and the vaginal smear method. Baumann and Steenbock (1932) studied the vaginal smear method on 45 rats. The animals were 4 weeks old and weighed 50 to 60 grams when placed on the vitamin A-free diet. Smears were examined daily. After cornification had persisted for 2 weeks, carotene was added to the diet. Of the 45 rats, 22 per cent showed cornified smears and stopped growing at the

same time, 18 per cent showed cornified smears for 3 to 7 days preceding stoppage of growth, and 33 per cent showed cornified smears for 7 to 14 days before cessation of growth. The majority of the rats stopped growing at the time xerophthalmia made its appearance. The above authors observed that a greater amount of carotene was needed to restore a normal smear picture than to cure xerophthalmia or to promote growth of the animals. They concluded that the vaginal smear method could be used as a quantitative method for determination of vitamin A-deficiency.

Beebe (1932), Aberle (1933), and Klussmann and Simola (1933) also concluded from their work that cornification of the rat vagina was a delicate indication of vitamin A-deficiency.

Mason and Ellison (1935) studied alterations in the vaginal smear during the development of vitamin A-deficiency. The interval between the first appearance of abnormal smears and complete continual cornification varied from a few days to a week. Estrous cycles could be followed until excessive cornification of the vaginal epithelium occurred. These authors also concluded that the change in the vagina was a much better index for detecting vitamin A-deficiency than xerophthalmia.

There are others who consider changes in the vaginal smear as an unsatisfactory index for vitamin A-deficiency. Coward (1929) and Coward, Morgan and Dyer (1930) did not

obtain cornified smears in the rat and maintained, therefore, that the vaginal smear was not a good index for vitamin A-deficiency. Thatcher and Sure (1932) tried to determine whether there were any metaplastic changes in the early stages of vitamin A-deficiency. Fifty-three rats were studied. At the start of the experiment the ages of the animals ranged from 28 to 38 days and they weighed from 41 to 84 grams. Metaplastic changes were observed, similar to those of Wolbach and Howe (1925). The estrous cycles were followed in 20 animals. Of these, only 9 showed a persistent cornified smear. Two animals which were autopsied at the sign of slight xerophthalmia had metaplastic changes of the posterior part of the tongue, but estrous cycles had been normal. The authors cited other instances where metaplastic changes were observed without disturbance of the estrous cycles. The authors concluded that persistence of the cornified stage of the estrous cycle in the rat was not a positive index of the first signs of vitamin A-deficiency. They also maintained that cessation of growth was not a reliable index for vitamin A-deficiency. They observed that 71 per cent of the vitamin A-deficient animals showed metaplastic changes before decline of body weight occurred.

Mason and Ellison (1935) made a careful study of the estrous cycle in the rat by supravital staining of the vaginal smears. The supravital staining was particularly useful when the smears were completely cornified. Animals with

marked xerophthalmia and loss in body weight showed atypical estrous cycles of 8 to 20 days in length. In animals that failed to obtain a weight of more than 100 grams while on the vitamin A-deficient diet, there was a complete absence of estrous activity. The authors observed in another group of animals that the quality of body growth, instead of the lack of vitamin A, was responsible for the lengthening of the estrous cycles. In this group the intake of food by the control animals was regulated so that the growth rate would be similar to that of the experimental animals. In both groups an increase in the length of the estrous cycle was observed.

In another experiment Mason and Ellison (1935) studied the combined effects of vitamin A-deficiency and inanition by placing the animals on a vitamin A-deficient diet in regulated amounts so that their body weights were maintained at 120, 150, 175, 225, and 250 grams. The frequency of the estrous cycles was roughly proportional to the weight of the deficient animals. With an increase in weight, the cycles became more regular. The authors also observed that xerophthalmia and abnormal cornification disappeared in vitamin A-deficient animals which were given daily doses of cod liver oil and whose body weights were kept at the same level as before. However, the estrous cycles were no more frequent than before treatment with cod liver oil. The prolonged estrous period was attributed to an indirect effect of the

vitamin A-deficiency as reflected in the nutritive state of the animals. The specific effect of vitamin A-deficiency was the abnormal cornification of the vaginal epithelium, and the indirect effect due to retarded body growth was manifested in the prolongation of the estrous cycle.

B. Overstimulation With Estrogen

The literature in past years has contained many reports on the histological response of tissues to estrogen stimulation. Zuckerman (1940) reviewed the literature and listed the tissue response to estrogen under three categories. The first is a property of most tissues and is manifested by a change in water content. The second type of response results from a direct or indirect effect of estrogenic stimulation upon various endocrine organs. The third type of response is cellular growth and is limited to certain tissues, such as those of the reproductive organs. The present review of the literature will be concerned with the third type of response. The changes in the uterus due to estrogen stimulation are usually epithelial proliferation of fibro-muscular growth, but this review will be concerned only with the epithelial response to chronic estrogenic stimulation.

Selye, Thomson and Collip (1935) were the first to study the effect of chronic estrone administration on the uterine epithelium. Eight female castrate rats were given

daily intraperitoneal injections of 30-60 ug of estrone over a period of 10 weeks. On histological examination of the uteri, 4 showed a more or less complete metaplasia of the epithelium into a stratified squamous epithelium with cornification, from which irregular buds penetrated down into the stroma. In another experiment animals were treated with moderate doses of estrone intraperitoneally and then 0.1 to 0.3 c.c. of 0.1 per cent estrone was placed in one horn of the uterus of each of 6 adult castrate rats. The animals were autopsied on the fourth day after filling the uterus. The estrone-treated horns showed signs of early metaplasia in 3 animals and marked metaplasia in 1 animal. The untreated horns were normal.

McEuen, Selye and Collip (1936) studied the effects of prolonged treatment with estrone on the uterus of the rat. Six castrate female rats, 3 to 4 months of age, received daily subcutaneous injections of 30 ug of estrone dissolved in corn oil for 331 days. The animals were autopsied 30 days after the last injection. During the latter 30 days the animals were injected with progesterone. On histological examination the authors observed extreme hypertrophic fibrosis which led to partial obliteration of the uterine cavity. They also observed squamous metaplasia in 5 of the animals.

McEuen (1936) investigated the effect of direct application of estrone to the uterine epithelium. A solution of

estrone, 1 mg. per c.c., was injected directly into the lumen of both uterine horns of 11 castrate female rats. The amounts injected varied from 0.05 c.c. to 1.25 c.c. After 103 days, partial metaplasia was present in 5 rats and extensive metaplasia in 3. In another series of 11 castrate female rats, estrone in corn oil was given subcutaneously in doses of 30 ug daily. Five animals autopsied on the 132nd day showed metaplasia of the uterine epithelium. Another group of 6 ovariectomized female rats was injected subcutaneously with a single dose of estrone, equal to the quantities injected directly into the uterine cavity of the animals of the first series. No metaplasia or any abnormality was present in the uterine horns after 103 days, in contrast to the effects of a continuous subcutaneous injection or one intra-uterine injection of estrone, which frequently produced metaplasia of the epithelium with down-growths into the stroma as already described.

In 1937, Nelson studied the effect of estrogenic hormones on the female guinea pig. Thirty-two guinea pigs were injected with estrogenic hormones from birth for periods varying from 2 to 10 months. Estrone in doses of 70 R.U. was injected every second day. Estrone benzoate and benzogynoestryl were injected twice a week in doses of 120 R.U. and 300 R.U. respectively. Fibromyomatous nodules were observed in the animals. Marked adenomatous hyperplasia of the endometrium was observed. In addition, extensive squamous

metaplasia of the tips and crypts of the glands was observed in the animals treated with the high doses of the hormones.

Zondek (1937) studied the changes in the rat uterus after prolonged treatment with estradiol benzoate. Castrate animals were injected twice a week with 5,000 M.U. of the hormone. All uteri did not react alike. In some the changes were severe, in others no changes were observed. In some of the uteri there was marked eosinophilic infiltration of the stroma. Other changes observed were localized absence of the epithelium, stratified cylindrical epithelium and partial to complete metaplasia of the epithelium. Zondek believed that an inflammatory factor played a determining role in producing the changes in the uterus. The author did not consider the changes to be precancerous lesions.

Loeb, Suntzeff and Burns (1938) made the first attempt to study the mechanism of metaplasia in the uteri of mice. They considered that there were two processes which led to a change in the luminal epithelium of the uterus. The first was a regenerative growth of the squamous cervical epithelium from the cervix into the uterus and the second was a true metaplasia of the columnar epithelium into one made up of several layers of cylindrical or cuboidal cells. Neither of these proceeded to actual keratinization. The authors believed that the regenerative origin of the squamous epithelium was the more frequent one. Uteri were

studied in mice which received various doses of estrogen and were autopsied from 1 to more than 20 months of age. The authors concluded that the incidence of stratified squamous epithelium in the uteri increased with the amount of estrogen injected. The evidence indicating the manner in which metaplasia of the luminal epithelium takes place is rather incomplete. The authors observed, as a first change, 2 or 3 layers of loosely arranged cylindrical cells in which mitoses were present. In other instances a layer of cuboidal cells developed under the original epithelium, and at other times 4 or 5 layers of cuboidal cells were seen. The superficial cells underwent hyalinization instead of keratinization. The cells that underwent this change to a transitional or a squamous epithelium acquired a great power for mobility, which was expressed by the squamous epithelium moving beneath the original epithelium and raising it from the connective tissue. Similar changes were observed in the glands of the uterus. The authors concluded that both the uterine and glandular epithelium have the potentiality of becoming transformed into transitional or stratified squamous epithelium, and of producing hyaline cells which they considered analagous to keratin formation in the vagina. They believed that the changes observed could not be considered as true precancerous conditions.

Korenchevsky, Hall and Burbank (1939) observed the effects of prolonged administration of sex hormones in fe-

male rats. Ovariectomy was performed at the age of 21 to 24 days. Doses of 18, 90 and 200 ug a week of estradiol dipropionate were used. The animals were injected for an average period of 190 days. With all doses, the weight of the vagina reached normal or even supranormal levels. Complete restoration of the uterus was not observed. The uterus was very fibrotic in structure. With the small doses the epithelial cells were high columnar and only 1 rat out of 8 contained a few patches of cells with squamous metaplasia. These authors considered this to be the first stage of precancerous change produced by estrogens. With 200 ug, metaplasia of the uterine epithelium was present in 40 per cent of the animals. Metaplasia was complete in some of the uteri involving the entire epithelial layer. The metaplastic changes in the uterine epithelium occurred more frequently and were more severe when androsterone was injected simultaneously with estradiol dipropionate.

Korenchevsky and Hall, in another experiment (1940), studied the pathological changes in the uterus of rats after prolonged administration of sex hormones. Ovariectomy was performed at the age of 21 to 23 days. The animals were injected weekly with: (1) 0.09 mg. of estradiol dipropionate; (2) 2.25 to 7.5 mg. testosterone propionate; and (3) estradiol dipropionate and testosterone propionate simultaneously. The majority of the animals were injected for a period of about 3 months. They concluded that squamous metaplasia may

occur with the same frequency in ovariectomized animals receiving estrogens alone as in those receiving the male hormone alone. The more severe conditions of squamous metaplasia occurred in the animals injected with both hormones. In another experiment the authors injected progesterone along with the other 2 hormones, which resulted in a much diminished development of metaplasia in the uterine epithelium. The dose of progesterone was 4.5 mg. per week. The authors contended that metaplasia of the uterine epithelium to the squamous-cell type (with or without keratinization) could be regarded as the first stage of the precancerous changes produced by estrogens. In the animals treated with estradiol dipropionate and testosterone propionate, the authors observed further pathological changes, i.e. branching of the uterine glands and formation of adenoma-like structures. The cells lining the adenoma-like structures varied in size and shape and contained nuclei of varying size. The lumina were filled with a secretion. In some cases the adenoma-like structures underwent squamous metaplasia.

Greene and Burrill (1941) stated that female rats given single large doses of estradiol dipropionate at birth, when examined 3 to 18 months later, had markedly enlarged oviducts showing inflammatory changes, small ovaries lacking corpora lutea, and uteri showing squamous metaplasia.

Weichert and Kerrigan (1942) observed changes in the uteri of the young of lactating rats treated with estrogen.

Lactating rats were treated daily, for the first 14 days after parturition, with 0.5 mg. of estrone. The young that were killed on the 14th day exhibited myometrial thickening and an indication of stratification in the lining epithelium. Very few glands were observed in these uteri.

Wilson (1943) investigated the effect of prepuberal treatment with estrogen on the reproductive organs. Thirty-two female albino rats received estradiol dipropionate in total doses ranging from 0.037 to 2.4 mg. The injections were started 24 hours after birth. Other animals were injected with a total dose of 1.2 mg. starting on the 5th, 10th, 15th, 20th and 30th and 40th days of age. The animals were injected over a period of 28 days and were not autopsied until they were 90 to 130 days old. Uteri of all the animals treated prepuberally, beginning on the 1st, 5th and 10th days, showed some abnormalities. However, the severity of damage tended to diminish as the age of the initial treatment increased. Injections on the 1st day showed pronounced effects. The glands were absent and the epithelium had undergone squamous metaplasia. Wilson maintained that metaplasia was induced by treatment during the 1st postnatal month, at least 3 months prior to examination of the uteri.

Hale (1944) observed changes in the uteri of animals treated daily with 0.01 mg. diethylstilbesterol for 14 days. The first injection was started within the first 2 weeks of age and the animals were reared to adulthood. The changes

observed were pyometra, endometritis, cystic glandular hyperplasia and squamous metaplasia of the uterine epithelium.

C. RELATIONSHIP BETWEEN VITAMIN A-DEFICIENCY AND ESTROGEN

The relationship between vitamin A-deficiency and estrogen in producing metaplastic changes in the uterus and abnormal cornification in the vagina is little understood. Eekelen (1931) observed that in castrate rats on a vitamin A-deficient diet, cornified cells appeared in the vaginal smear before the animals lost weight. Small doses of carotene cured the condition in a few days.

Mason and Wolfe (1935) studied the relation of castration to vitamin A-deficiency in the albino rat. They were particularly interested in xerophthalmia and in the vaginal changes that occurred in vitamin A-deficient animals. The animals were 19 to 26 days of age and weighed 40 grams at the start of the experiment. The average time required to induce cornification was 22.2 days, while the average time for xerophthalmia was 37.3 days. The authors concluded that castration caused no significant difference in the time of appearance of continuous vaginal cornification or of xerophthalmia in vitamin A-deficient rats, and that vaginal cornification could be detected sooner than xerophthalmia.

Sherwood, Brend and Roper (1936) studied the changes in the vaginal epithelium of the rat on a diet containing an

excess of vitamin A. The vaginal smears were observed to be abnormal within 2 days following carotene administration. The smears did not go from the nucleated cell stage to the cornified stage, but consisted entirely of nucleated epithelial cells. The estrous cycle did not return to normal until 20 days after the excess carotene feeding was discontinued. The authors maintained that the results were due to active cell growth as indicated by the large number of nucleated cells in the smears. Since normal cycles were observed in the controls, the changes in the smears of the experimental animals must have been brought about by the large amount of vitamin A given.

McCullough and Dalldorf (1937) studied the relationship of estrone and vitamin A-deficiency in producing metaplastic changes in the uterus of the rat. Castrated immature females were used and were divided into 3 groups of 4 animals each: (1) the 1st group was placed on a vitamin A-deficient diet for 34 to 70 days and received 25 ug of estrone daily; (2) the 2nd group was placed on a normal diet and received 25 ug of estrone daily for 29 to 37 days; (3) the 3rd group was placed on a deficient diet for 32 to 62 days, but did not receive estrone. Metaplastic changes occurred regularly in the endometrium if estrone was given and the diet was deficient. However, the animals on the normal diet and receiving the hormone daily, and the rats on the deficient diet only did not show any indication of meta-

plasia in the endometrium. The authors maintained that the intake of vitamin A prevented metaplasia and that estrone was not concerned with its formation in the animals that were on a normal diet and receiving estrone daily. In the group of animals on a deficient diet the authors believed that metaplasia did not occur because the period of the deficient diet was too short. The authors used as criteria of metaplasia the appearance of keratinized epithelium, the formation of flat, non-nucleated surface cells and the presence of keratohyaline granules in the epithelium. The results indicated that the factor indispensable to metaplasia was the deficiency of vitamin A. The authors maintained that vitamin A-deficiency was the primary condition essential for metaplasia and that estrone acted solely as a secondary factor.

Hume, Burbank and Korenchevsky (1939) observed metaplastic changes in the male reproductive organs when the rats were deprived of vitamin A and treated with estrogen. The changes were more severe with the dual treatment than with either treatment alone.

Burrill and Greene (1941) observed that vitamin A-deficiency did not increase the sensitivity of the castrate rat vagina to administered estrogen. The castrate animals were divided into 2 groups. The 1st group was fed a normal diet plus an excess of vitamin A daily, the 2nd group was on a vitamin A-deficient diet. At weekly intervals the

groups were tested for their response to 1.0 ug estrone after a priming dose of 5.0 ug. Excess vitamin A did not inhibit the response of the vagina to administered estrogens in the castrate animal. The animals on the vitamin A-deficient diet did not exhibit an increased responsiveness to injected estrogen.

Kahn and Bern (1950) studied the antifolliculoid activity of vitamin A in the rat. Castrate females were estrogenized by subcutaneous implantation of estradiol pellets. After cornified smears were obtained, the animals were treated with sesame oil or with vitamin A in sesame oil applied intravaginally in 0.05 ml. daily doses. Animals that were treated with vitamin A exhibited a definite alteration of the keratinization produced by estrogen. The smears contained ovoid and round cells with vesicular nuclei and many large vacuoles in their cytoplasm. The animals that were treated with sesame oil showed no inhibition of the estrogen induced keratinization.

STATEMENT OF THE PROBLEM

From the literature it is evident that epithelial metaplasia in the uterus of the rat is not clearly understood.

The changes reported in the uterus due to vitamin A-deficiency, as reviewed in the previous section, have been observed only in the intact animal. Since the normal growth

and differentiation of the uterus depends on the endocrine activity of the ovaries, and since estrogen is also capable of producing uterine metaplasia, the question arises as to what role endogenous estrogen plays in the development of metaplastic changes in the uteri of vitamin A-deficient rats.

As indicated above, metaplastic changes in the uterus have also been produced by chronic estrogen treatment. Such observations, however, have been made either on animals autopsied after prolonged treatment or on animals which were injected for a short time prepuberally and which were not autopsied for several months thereafter. The results obtained by previous investigators have left several important questions unexplained. First, is metaplasia a gradual process or a rapid one which takes place a short time after the beginning of estrogen treatment? Secondly, from the literature it is also evident that the age of the animal at the start of estrogen treatment is an important factor in the production of metaplastic changes in the uterus. This age factor, however, has seldom been considered by previous investigators. Finally, no adequate comparison has been made concerning the histological differences between the metaplastic processes resulting from estrogen treatment and those resulting from vitamin A-deficiency.

The present investigation attempts to answer the above questions by: (1) a histological study of the uteri of rats

ovariectomized on the 20th day of age and subsequently raised on a vitamin A-deficient diet, and (2) by a similar study of the uteri of intact rats treated with estrogen beginning at the 7th day of age, and of intact and ovariectomized rats treated with estrogen beginning at the 21st day of age. Details of the experimental procedures will be given below.

MATERIALS AND METHODS

Two-hundred female rats of the Wistar strain were used in these experiments. The majority of the animals were born and raised in our colony. Ten rats used in the estrogen experiments were purchased from the Hamilton Laboratories. The animals were weaned on the 20th day, weighing approximately 35 grams at that time. Three to four rats were usually kept in one cage. In the case of pair-fed animals, however, only 1 rat was kept in a cage. Except for the pair-fed rats, the animals on a vitamin A-deficient diet[1] were given the diet ad libitum. The deficient diet of the estrogen treated animals and of some of the vitamin A-deficient animals was supplemented with 250 I.U. of vitamin A which was given

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

orally once a week. The vitamin A concentrate[2] was dissolved in cottonseed oil and 0.1 c.c. of solution contained 250 I.U. of vitamin A.

At the conclusion of each experiment, the rats were killed with chloroform vapors and were autopsied immediately. The uteri were fixed for 12 to 24 hours in Zenker's solution.[3] The tissues were subsequently washed for 12 hours. After dehydrating in ethyl alcohol and embedding in paraffin, serial sections were cut 6 u in thickness. Every 10th section was mounted and stained with Weighert's iron-hematoxylin and counterstained with eosin.

A. Vitamin A-Deficiency Experiments

Mothers were placed on a vitamin A-deficient diet on the day a litter was born. Males were removed from the litter on the 2nd day after birth. Three to 5 females were kept with each mother during the first 20 days. Eighty

[2]Vitamin A Ester Concentrate, General Biochemicals, Inc., Chagrin Falls, Ohio.

[3]Zenker's solution:
100 c.c. of water
7 gm. of mercuric chloride
1 gm. of sodium sulfate
2.5 gm. of potassium bichromate
5 c.c. of acetic acid

animals raised in the above manner were divided into 4 groups.

In group 1, 30 rats were kept intact and were continued on the vitamin A-deficient diet. In group 2, 10 animals were treated as in group 1 except they received a dietary supplement of vitamin A as explained above. Group 3 consisted of 30 rats which were bilaterally ovariectomized on the 20th day and were continued on the vitamin A-deficient diet. In group 4, 10 rats were treated the same as in group 3 except that they received a dietary supplement of vitamin A as explained above. All the rats in the 4 groups were examined daily for external manifestations of vitamin A-deficiency and were weighed periodically during the experiment. Five animals in group 1 and 5 animals in group 3 were autopsied during the 8th, 9th, 10th, 11th, 12th and 13th weeks. Several animals of groups 2 and 4 were autopsied during the 8th to 13th week. Uterine specimens were taken for histological study.

In order to determine whether the daily food intake of the vitamin A-deficient animals was an important factor in the appearance of symptoms, 5 of the ovariectomized vitamin A-deficient rats and 5 of the ovariectomized rats on a diet supplemented with vitamin A were pair-fed during the experiment.

B. Estrogen Experiments

A total of 120 animals were used in these experiments. One-hundred rats received a single subcutaneous injection of 2 mg. estradiol dipropionate[4] per week and were divided into 3 groups. The remaining animals were placed in group 4.

In group 1 (55 rats) the injections were started on the 7th day after birth. Five animals were autopsied every 3 days from the 3rd to the 27th day after receiving the 1st injection, 5 rats were autopsied on the 33rd day, and 5 on the 39th day after the 1st injection. In group 2, 36 animals were bilaterally ovariectomized on the 20th day of age. The injections were started on the following day. Four or 5 animals were autopsied every 3 days from the 12th to 27th day after the 1st injection. Four animals were autopsied on the 33rd day and 5 on the 39th day after the 1st injection. Group 3 consisted of 9 normal animals and injections were started on the 21st day of age. Five animals were autopsied 21 days later and 4 were autopsied 39 days later.

In group 4, 20 rats were used as controls. Several

[4]The estradiol dipropionate used in these studies was supplied through the courtesy of the Ciba Pharmaceutical Products, Inc., Summit, N. J.

rats were autopsied on the 7th, 13th, 19th, 28th, 45th and 60th day of age. Six control rats were ovariectomized on the 20th day of age. Three of these were autopsied on the 40th day and 3 on the 60th day of age. Uterine specimens were taken for histological study.

OBSERVATIONS

In order to avoid repetition of the findings, most of the observations will be presented in narrative form. The observations were made, as mentioned under "Materials and Methods", on animals autopsied on different days during the experiment.

A. Vitamin A-Deficiency Experiments

1. Gross pathology of the ovariectomized and intact vitamin A-deficient rats.

The external manifestations due to vitamin A-deficiency in the rat have been reported many times. The following is a brief account of the changes which occurred during the course of the present experiments.

The first external sign due to vitamin A-deficiency was alteration of the vaginal smear. In the intact and ovariectomized rats, cornified cells appeared in excess in the

smears during the 6th week of age. The abnormal cornification of the vagina was much more severe in the intact than in the ovariectomized rats. In both groups, encrusted eyelids appeared during the 7th week of age.

Most animals developed difficulty in breathing during the 9th week. A few developed this condition much earlier and died soon after. The mortality rate was high apparently because of the respiratory disorders that developed.

In the 10th week, the intact as well as the ovariectomized rats became irritable and maintained the typical humped posture exhibited in vitamin A-deficiency. In the later stages of the experiment (12th and 13th weeks) the animals of both groups had difficulty in moving about in the cages.

Intact and ovariectomized rats autopsied during the 10th through the 13th week of age exhibited a depletion of fat throughout the body. The ureters and bladders of a number of animals autopsied in the 11th, 12th and 13th weeks were greatly distended with urine. The uteri of the ovariectomized animals were greatly diminished in size and were difficult to locate at autopsy, whereas the uteri of the intact animals were normal in gross appearance.

2. Histological observations on the uteri of intact vitamin A-deficient rats.

The metaplastic changes observed were similar to the ones that have previously been reported by Wolbach and Howe (1925). The following is a brief account of these changes as observed during the present experiment.

The first indication of uterine metaplasia appeared in 2 of 5 rats autopsied during the 8th week of age. All animals autopsied after the 8th week showed some degree of metaplasia. The first indication of metaplasia was a clumping of the cells of the luminal epithelium (fig. 1). This mass of cells formed what appeared to be a syncytium and extended down into the stroma of the underlying lamina propria (fig. 2) undermining the original lining epithelium. Keratinization of this stratified epithelium, however, was not observed.

Stratified squamous keratinized epithelium appeared in some of the uterine glands during the 10th week and the lumina were filled with keratinized material. Squamous metaplasia was present in some glands next to apparently normal appearing glands and luminal epithelium (figs. 3, 4, 5 and 6).

3. Histological observations on the uteri of ovariectomized vitamin A-deficient rats.

In contrast to the above results, metaplastic changes were not observed in the luminal and glandular epithelium of the uteri of ovariectomized vitamin A-deficient rats during any interval up to the 13th week (figs. 7 and 8). The uteri were small with a narrow lumen. Luminal cells were low in height and glands were few in number in some of the uteri.

4. Observations on intact and ovariectomized rats receiving regular supplements of vitamin A.

The animals receiving supplements did not show any external manifestations of vitamin A-deficiency. Histological examination of the uteri revealed no metaplastic changes of the luminal or glandular epithelium.

5. Observations on the pair-fed animals.

The ovariectomized vitamin A-deficient rats which were pair-fed showed the same external manifestations as were previously described, such as abnormal vaginal cornification, encrusted eyelids and humped posture. On histological examination of the trachea of these animals, metaplastic

changes were observed in the lining epithelium. Ovariectomized rats on the supplemented diet, however, did not exhibit any external manifestations of A-deficiency nor metaplasia of the trachea. The animals on the supplemented diet gained weight steadily, while the animals on the deficient diet stopped gaining weight after they reached a weight of approximately 125 grams. The amount of food given per day to the rats on a supplemented diet was equal to the amount of food consumed by the A-deficient animals the previous day. At autopsy the rats on a supplemented diet weighed approximately 25 to 30 grams more than the rats on the deficient diet. On microscopic examination of the uteri, metaplastic changes were not observed in rats on the deficient diet or in those on the supplemented diet.

B. Overstimulation With Estrogen

Data for rats treated with estrogen are shown in Table I.

1. General histological observations on uteri of intact and ovariectomized rats treated with estrogen beginning on the 7th and 21st days of age.

The histological observations on uteri of rats not showing squamous metaplasia will be considered first. The luminal cells of the uteri were, for the most part, hyper-

trophied and a distinct basement membrane separated them from the underlying connective tissue. In a few uteri the lining epithelium consisted of low columnar cells while in others the epithelium appeared pseudostratified with some vacuolated cells. In all of the uteri the endometrial glands were few in number.

In some of the uteri there was a marked thickening of the stroma and myometrium which resulted in almost obliterating the lumina, while in others the stroma and muscle layers were thin and the lumina were large. Eosinophiles and neutrophiles were present in the stroma and among the muscle cells of the uteri. The number of white blood cells was more pronounced in the animals treated for a long period of time. Pyometra, which is a destruction of the lining epithelium and accumulation of pus in the lumen of the uterus, was observed in a few of the animals.

2. Histological observations of uteri of intact rats treated with estrogen beginning at the 7th day of age.

Squamous metaplasia was not present in the uteri of rats autopsied from the 3rd through the 15th day after the first injection when treatment was started at the 7th day of age. The changes were similar to those mentioned under general observations (1). Pyometra was present in one of the animals autopsied on the 15th day.

Four of the rats autopsied on the 18th day had foci of stratification in the lining epithelium (fig. 10). The cells were hypertrophied, but squamous formation of the superficial cells was not observed. Keratinization was not observed in any of the foci. In some portions of the uterus the stratification gave the appearance of papillary formation.

Extensive squamous metaplasia of the uterine epithelium was observed in 8 of the 10 rats autopsied on the 21st and 24th days (figs. 14 and 15). The posterior halves of the uteri had stratified squamous keratinized epithelium lining the lumina. This portion of the uteri contained desquamated epithelial cells, eosinophiles and neutrophiles. The stratified squamous epithelium of the uterus was continuous with the stratified squamous epithelium of the cervix. For the most part, the lumina of the anterior half of the uteri were filled with what appeared to be granulation tissue which consisted of fibroblasts, blood vessels, eosinophiles and neutrophiles (fig. 16). In the anterior tip of the uterine horns of the animals autopsied on the 21st day, the lining epithelium was destroyed and great numbers of neutrophiles were present in the lumina and surrounding connective tissue. The anterior tips of the uterine horns of 2 animals autopsied on the 21st day were cystic. The cysts were lined with simple squamous epithelium and many neutrophiles were present in the lumina and surrounding

tissue. The cells of the uterine epithelium of the 2 rats that did not show squamous metaplasia were similar to those described under general observations.

Squamous metaplasia was present in 6 of the 10 animals autopsied on the 27th and 33rd days. One rat autopsied on the 27th day and 1 on the 33rd day had extensive squamous metaplasia which was similar to that in the animals autopsied on the 21st and 24th days. The remaining 4 rats contained many foci of stratified squamous epithelium (figs. 11, 12 and 13). The foci consisted of clumps of epithelial cells which had undermined the original epithelium, separating it from the underlying connective tissue. In some instances the original epithelium rested on a layer of stratified epithelium. In more advanced conditions the original cells had been shed into the lumina of the uteri, the superficial cells of the new epithelium were squamous in character, and in some foci keratinization was present.

Four of the rats autopsied on the 39th day had uterine metaplasia. In 1 of the 4 animals the upper half of the uterine epithelium was destroyed and many neutrophiles were present in the lumen and surrounding connective tissue. The posterior half of the uterus was lined with thin stratified squamous epithelium. The uterine epithelium of the remaining 3 rats contained multiple foci of squamous metaplasia as described in the animals autopsied on the 27th and 33rd days.

3. Histological observations on the uteri of ovariectomized rats treated with estrogen beginning on the 21st day of age.

Squamous metaplasia was observed in only 2 of the 36 rats autopsied from the 12th to the 39th day when the 1st injection was given on the 21st day. Multiple foci of squamous metaplasia were observed which were similar to the ones described in the animals treated for the first time on the 7th day of age and autopsied on the 27th and 33rd days. The uteri of the remaining animals were similar to those described under general observations. Pyometra was present in the uterus of 1 of the rats autopsied on the 21st day.

4. Histological observations on the uteri of intact rats treated with estrogen beginning on the 21st day of age.

Squamous metaplasia was not observed in any of the intact animals autopsied on the 21st to 39th days after the 1st injection when injection was started on day 21 (fig. 17). The uteri were similar to those described under general observations. Pyometra was present in 1 of the animals autopsied on the 39th day.

5. Histological observations on the untreated control animals.

Metaplastic changes were not observed in the uteri of the ovariectomized or intact control rats (fig. 18).

Table I

Metaplasia in the Uteri of Rats
Treated With 2.0 mg. Estradiol Dipropionate
per Week Beginning on the 7th and 21st Days of Age

Age at start of treatment	No. of animals	Intact or castrate	Days after 1st inj. autopsied	No. of animals showing metaplasia	Extent of metaplasia
7 days	25	intact	3-15	0	-
" "	5	"	18	4	±
" "	5	"	21	4	+++
" "	5	"	24	4	+++
" "	5	"	27	4	++
" "	5	"	33	2	+++
" "	5	"	39	4	++
21 days	9	intact	21&39	0	-
" "	13	castrate	12-18	0	-
" "	6	"	21	1	+
" "	4	"	24	1	+
" "	4	"	27	0	-
" "	4	"	33	0	-
" "	5	"	39	0	-

DISCUSSION

A. Vitamin A-Deficiency

Many of the manifestations of vitamin A-deficiency are secondary to changes in the epithelial cells, and in general, the function of vitamin A appears to be that of a regulator of certain metabolic processes which are essential in maintaining the normal structure and formation of epithelia. When the normal metabolic processes are disrupted, due to a lack of vitamin A, a change in the epithelial cells takes place which may result in a stratified keratinized epithelium.

The metaplastic changes observed in the uteri of the vitamin A-deficient rats were similar to those reported by Wolbach and Howe (1925). In brief, such changes consisted of clumping of the cells of the uterine epithelium forming what appeared to be a syncytium, and the extension of the epithelial-like cells down into the stroma of the endometrium. The luminal epithelium became stratified, but no indication of keratinization was observed. However, keratinization did appear in the uterine glands. Of major interest, on the other hand, was the observation that in the ovariectomized vitamin A-deficient rats no metaplasia of the uterus developed.

McCullough and Dalldorf (1937) have maintained that

squamous metaplasia and keratinization of the uterus result from a local vitamin A-deficiency irrespective of any other factors. The present observations do not agree with their conclusions. The results of the present investigation indicate that the ovaries also have an important role in producing metaplasia in the uteri of vitamin A-deficient rats. Before the manifestations of vitamin A-deficiency can be expressed on the uterus, the ovaries, by their endocrine function, have to stimulate normal growth and function of the organ. Of the ovarian hormones it is probably estrogen that is concerned with the metaplastic changes in the uterine epithelium, since the main effect of progesterone on the epithelial cells of the uterus is to stimulate them to secrete, whereas that of estrogen is to stimulate mitosis and cell growth. There is a possibility that metaplastic changes would have occurred in the uteri of ovariectomized rats if they had been kept on the deficient diet for a longer time, but the mortality rate was high, and it was only with great difficulty that animals could be maintained on the deficient diet for as long as 13 weeks.

The vagina, although under the same ovarian influence as the uterus, reacted differently to vitamin A-deficiency. Abnormal cornification appeared in the ovariectomized as well as in the intact animals. The reason for this difference is not definitely known at present, although there is evidence to indicate that it may be a result from the fact

that the epithelium and embryological origin of the uterus and vagina differ. The uterus is lined with columnar epithelium and is derived from mesoderm while the vagina is lined with stratified epithelium and is derived, for the most part, from entoderm. It should be noted that the majority of areas that show metaplastic changes or abnormal cornification due to vitamin A-deficiency are already stratified or have a tendency toward stratification, such as vagina, bladder, skin and trachea. In general, epithelia derived from entoderm are more sensitive to vitamin A-deficiency than those derived from mesoderm.

A further explanation for the dissimilar reaction may result from the fact that: (1) the vagina is much more sensitive to estrogen than the uterus and (2) a small amount of estrogen is produced by the adrenals in the ovariectomized rat. Marrion and Parks (1930) reported that, in the mouse, 200 times the amount of estrogen which would produce complete vaginal estrus (one mouse unit) was necessary for a full estrous reaction of the uterus, in 50% of the animals, 72 hours after the 1st injection of estrogen. Szorka and Kuntz (1938), working with the rat, observed that 4.8 ug of estrone were necessary to produce a typical estrous uterus 44 to 50 hours after the injection, while the vagina responded to one fifth this amount. Parks (1945) summarized the findings which indicated that the secretion from adrenal glands has a weak estrogenic activity. Some of the findings

were: (1) that after complete ovariectomy, a subdued vaginal rhythm was present in the mouse and (2) that the opening of the rat vagina is only slightly delayed after ovariectomy or adrenalectomy, but if both the adrenal glands and ovaries are removed the opening may be delayed for 2 or more months. Pliske (1953) observed that hog adrenal cortical extracts stimulated cornification of the vaginal epithelium of ovariectomized adult rats.

The estrogen activity from the adrenal glands may be sufficient in ovariectomized rats to stimulate the vaginal epithelium and act as a precipitating factor in bringing about abnormal cornification in vitamin A-deficient rats. On the other hand, the uterus, having a low sensitivity to estrogen in comparison to the vagina, is not stimulated by the adrenal estrogen and vitamin A-deficiency by itself cannot produce uterine metaplasia in the ovariectomized animals. It would be interesting to determine whether abnormal cornification would occur in the vaginal epithelium of vitamin A-deficient rats after adrenalectomy.

One problem that arises in connection with vitamin deficiency is that of distinguishing between the effects of the vitamin deficiency and the indirect effects of growth retardation due to anorexia or other causes. In the present observations, however, the pair-fed animals on the supplemented diet did not exhibit any external manifestations of vitamin A-deficiency and a histological study of the trachea

did not reveal any metaplastic changes. Since the animals on the deficient diet did show external symptoms of vitamin A-deficiency and metaplastic changes were present in the trachea, it can be concluded that the metaplasia observed was due to vitamin A-deficiency and not to indirect effects of the deficiency.

B. Overstimulation With Estrogen

The present experiments have demonstrated a marked difference in response to overstimulation with estrogen between the animals treated for the 1st time on the 7th day of age and those treated on the 21st day. Twenty-two of 30 animals treated on the 7th day developed pronounced metaplastic changes from the 18th to 39th day after the 1st injection. Only 2 of 45 animals treated for the 1st time on the 21st day, however, showed metaplastic changes in the uterus in this same time interval.

Since the cytological processes involved in producing squamous metaplasia of the uterine epithelium were described separately under "Observations", the following is a brief summary of the changes that took place. The first evidence of squamous metaplasia was a clumping of the cells of the lining epithelium. The mass of epithelial cells extended towards the lumina of the uteri and also laterally from the point of origin, undermining the original epithelium between

the basement membrane and the luminal cells. The superficial cells of this new cell growth became squamous and keratinization occurred. It was not unusual to see cells of the original epithelium resting on the stratified squamous epithelium. By the above process the entire luminal epithelium was replaced by a stratified squamous keratinized epithelium.

Loeb, Suntzeff and Burns (1938) suggested that in most instances uterine metaplasia occurred in the mouse by direct extension of the stratified cervical epithelium. They felt that ulceration of the uterine epithelium preceded the regenerative growth from the stratified epithelium of the cervix. The replacement epithelium did not undergo keratinization, but hyalinization did occur. The present observations have shown that squamous metaplasia of the uterine epithelium of the rat appears by multiple foci and that the replacement epithelium does become keratinized.

The results indicate that age of the animal at the start of the estrogen treatment is an important factor in determining how rapidly squamous metaplasia of the uterine epithelium occurs. The difference in response between the 2 age groups indicates that the sensitivity of the rat to estrogen decreases with an increase in age of the animal. This difference in sensitivity of the uterus to sex hormones has been observed by previous investigators. Wilson, Hamilton and Young (1941) observed uterine metaplasia in rats

treated with testosterone propionate for the 1st time on the 5th or 10th day of age, but changes were not observed when the injections started on the 15th, 20th or 30th days. The injections were given 3 times weekly for 4 weeks and the animals were autopsied 2 to 4 months after the last injection. Wilson (1943) observed similar results when female rats were treated prepuberally with estrogen. Weichert and Hale (1943) observed that metaplasia of the ducts of Cowper's glands, prostatic utricle and caudal portions of the ducts of the seminal vesicles varied with the age of the rat at the time when injections were first begun. The animals received daily injections of 0.1 mg. of diethylstibestrol for 14 days beginning on the 7th, 14th and 21st days of life.

The morphological changes that take place in the uteri of rats with increase in age may play a role in determining how rapidly metaplasia occurs after estrogen treatment. Wiesner (1934), in studying prepuberal uteri of rats, observed that in the new-born the uteri were small embryonic structures with narrow lumina lined with low epithelium, and the stromal and muscle cells were not completely differentiated. Within 2 weeks the lumina were lined with columnar epithelium, glands were present, stromal cells were differentiated from the muscle cells and the myometrium contained 2 distinct layers. Since there is this change in morphology of the uterus associated with increase in age, it seems that the growth stimulating effect of estrogen would be much more

effective in producing metaplasia in a undifferentiated uterus than in a uterus that is completely differentiated.

The present results also indicate that the transformation of the columnar epithelium of the uterus to a stratified squamous keratinized epithelium does not take place within a short time after the 1st injection. Metaplastic changes were not observed until the 18th day after the 1st injection. However, once metaplasia started, the process was rapid and the entire uterine epithelium was changed over to a stratified squamous keratinized epithelium within several days.

The inflammatory reaction that occurred in the uteri following chronic estrogen treatment was considered by Zondek (1937) to play the determining role in producing metaplasia of the uterine epithelium. Gardner and Allen (1937), using mice 38 to 42 days old, removed the anterior three fourths of both uterine horns and transplanted them into the inguinal region. The animals then received weekly injections of 500 I.U. of estrogen over a period of 4 to 8 months. At autopsy the uterine grafts were distended and filled with a clear liquid. The sections contained few leucocytes and the epithelium consisted of cuboidal and low columnar cells. In none of the grafts was there evidence of leucocytic invasion or pyometra. Pyometra, or evidence of inflammation, was present in the uterine stumps. The observations showed that pyometra did not develop in the grafts removed from their

normal connection with the cervix and the authors concluded that prolonged treatment with estrogen may induce changes in the uterus which favor infection, but pyometra cannot be contributed to a direct action of estrogen.

In the present study, metaplastic changes appeared in the uterine epithelium without the presence of inflammation. It was not until stratified squamous keratinizing epithelium was present that an inflammatory reaction was observed in some of the uteri. In a few of the animals the lumina were filled with granulation tissue or fibrous tissue which was apparently formed after the destruction of the stratified squamous epithelium by the inflammation. The formation of granulation tissue and finally of fibrous tissue is considered to be an attempt on the part of the body to repair the damage done by inflammation. From the present study, it can be concluded that inflammation cannot be considered as playing the determining role in producing uterine metaplasia.

C. Miscellaneous Considerations

Bern (1952) studied metaplasia in the male reproductive system of the rat and mouse produced by vitamin A-deficiency and estrogen treatment and observed that the metaplasia produced by estrogen could be distinguished from that produced by vitamin A-deficiency on the basis of two principal points:

(1) that metaplastic growth extends towards the lumen in the former and away from the lumen in the latter and (2) from a histochemical study, that alkaline phosphatase is present in the basal cells only after estrogen treatment. In the present study it was also observed that the metaplastic changes produced in the uteri due to vitamin A-deficiency and overstimulation with estrogen differ in two essential ways: (1) the manner of growth of the replacement epithelium and (2) the extent of keratinization. In the vitamin A-deficient animals, the clumping of the luminal cells extended down into the underlying lamina propria, whereas in the estrogen treated animals the mass of luminal cells extended towards the lumina of the uteri. Squamous keratinized epithelium of the luminal cells of the deficient rats was not observed while in the estrogen treated animals keratinization was observed. Keratinized epithelium was present in the endometrial glands of the vitamin A-deficient animals, while in the estrogen treated rats the glands were few in number and evidence of keratinization was not observed. Squamous metaplasia of the uterine epithelium was much more extensive in the estrogen treated animals than in the vitamin A-deficient rats.

Since there is a difference in the uterine metaplasia produced by vitamin A-deficiency and overstimulation with estrogen, a histochemical study of the 2 processes might help to clarify the relationship between estrogen and vitamin

A-deficiency in producing the metaplastic changes.

The relation of epithelial metaplasia to neoplasia has been of interest for many years and various investigators have proposed and opposed the idea that metaplastic changes are precancerous. Korenchevsky and Hall (1940) considered the metaplastic changes that occurred in the uterus of the rat after estrogen treatment to be precancerous. Likewise, Patch (1948) studied the metaplastic changes that occurred in the luminal epithelium of the human urinary tract and considered them to be precancerous. On the other hand, Zondek (1937) considered the metaplastic changes that occurred in the uteri of rats treated with estrogen as not being precancerous. Auerbach and Pund (1945) did not consider the metaplastic epithelium that appeared in the human cervix uteri as having any relation to a malignant disease. Bullock, Hummen and Kohler (1952) reported that there was no evidence that squamous metaplasia was a precursor to squamous cell carcinoma of the thyroid gland. Since there is this difference of opinion, a more thorough study of the relation of the processes is needed.

SUMMARY

1. The two methods used to study epithelial metaplasia in the uterus of the rat were vitamin A-deficiency and overstimulation with estrogen.

2. The metaplastic changes observed in the uteri of vitamin A-deficient intact rats were similar to those reported by previous authors. These consisted of stratification of the luminal epithelium and keratinization of the glandular epithelium. No metaplastic changes were found, however, in the uteri of vitamin A-deficient rats which had been ovariectomized. This difference in response indicates that ovarian estrogen plays an important role in producing uterine metaplasia and that vitamin A-deficiency should not be considered the sole factor.

3. Abnormal cornification of the vagina due to vitamin A-deficiency occurred in the intact as well as in the ovariectomized animal. The possibility that estrogen from the adrenal cortex is involved in producing such changes in the ovariectomized animal is discussed.

4. A marked difference in response to overstimulation with estrogen was observed between the animals treated for the 1st time on the 7th day of age and those treated on the 21st day. Twenty-two of 30 animals treated on the 7th day developed pronounced metaplastic changes from the 18th to the 39th day after the 1st injection. Only 2 of 45 animals

treated for the 1st time on the 21st day of age, however, showed metaplastic changes in the uterus in this same time interval.

5. The observations indicate that estrogen-induced uterine metaplasia begins as numerous independent foci and not as an extension of the stratified cervical epithelium. The metaplastic changes do not take place within a short time after the 1st injection, but once the metaplastic changes begin the entire uterine epithelium is replaced by stratified squamous keratinized epithelium within several days.

6. Some investigators have claimed that the presence of an inflammatory response was an important factor in producing uterine metaplasia. Since the majority of the animals in this experiment did not clearly have an inflammatory response accompanying uterine metaplasia, it cannot be considered as playing an important role in producing metaplasia of the uterus.

7. Comparison of the metaplastic changes in the uterus due to vitamin A-deficiency and those due to overstimulation with estrogen revealed that there was a considerable difference in cytologic appearance. In the vitamin A-deficient rats, the luminal cells showing the first indication of metaplasia extended into the lamina propria, while in the estrogen-treated rats the metaplastic cells bulged into the lumen of the uterus. Keratinization of the lining epithelium

of the uterus in the vitamin A-deficient rats was not observed; however, keratinization was observed in the epithelium of the uterine glands of these animals. The estrogen-treated animals exhibited opposite effects. The lining epithelium became keratinized, but the glandular epithelium did not. It seems probable, therefore, that estrogen and vitamin A-deficiency produce uterine metaplasia by different mechanisms, the details of which remain obscure.

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PLATE I

Explanation of Figures

Figs. 1 and 2 Portion of the endometrium of intact rats showing clumping of the luminal cells, forming what appears to be a syncytium, and the extension of the cells into the stroma of the endometrium. The animals were on a vitamin A-deficient diet and autopsied during the 8th and 9th weeks of age respectively.

PLATE I

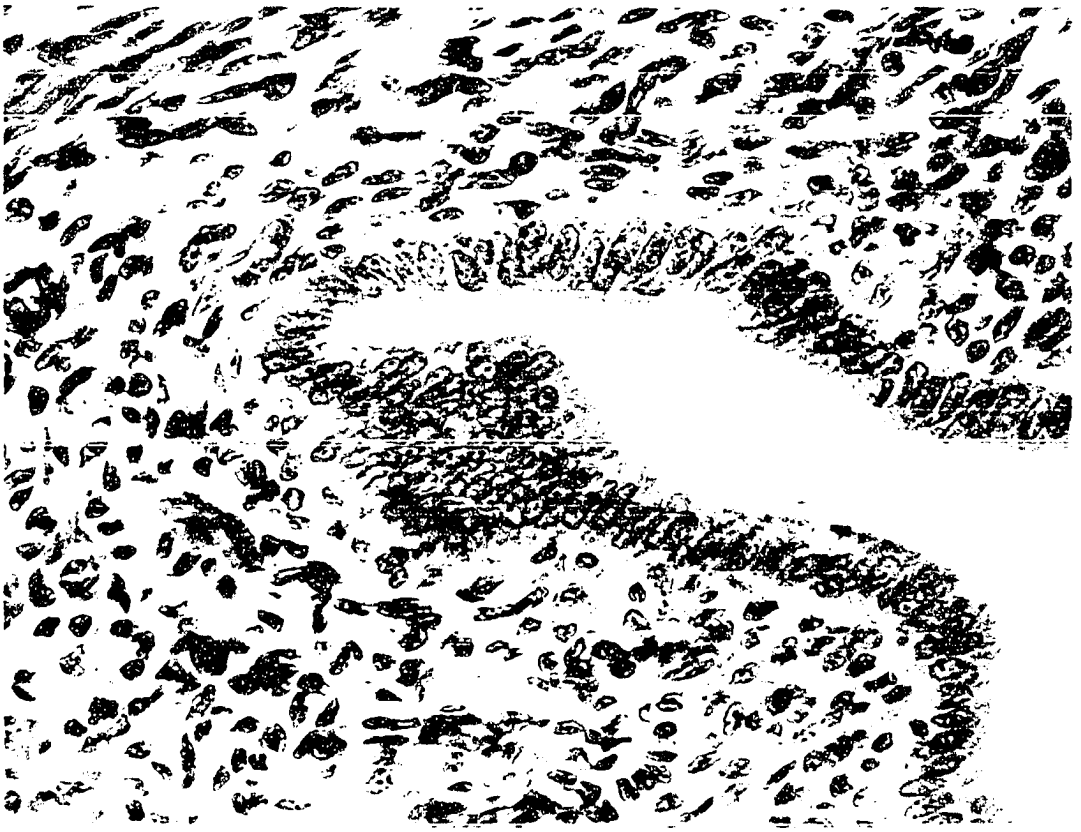

Fig. 1

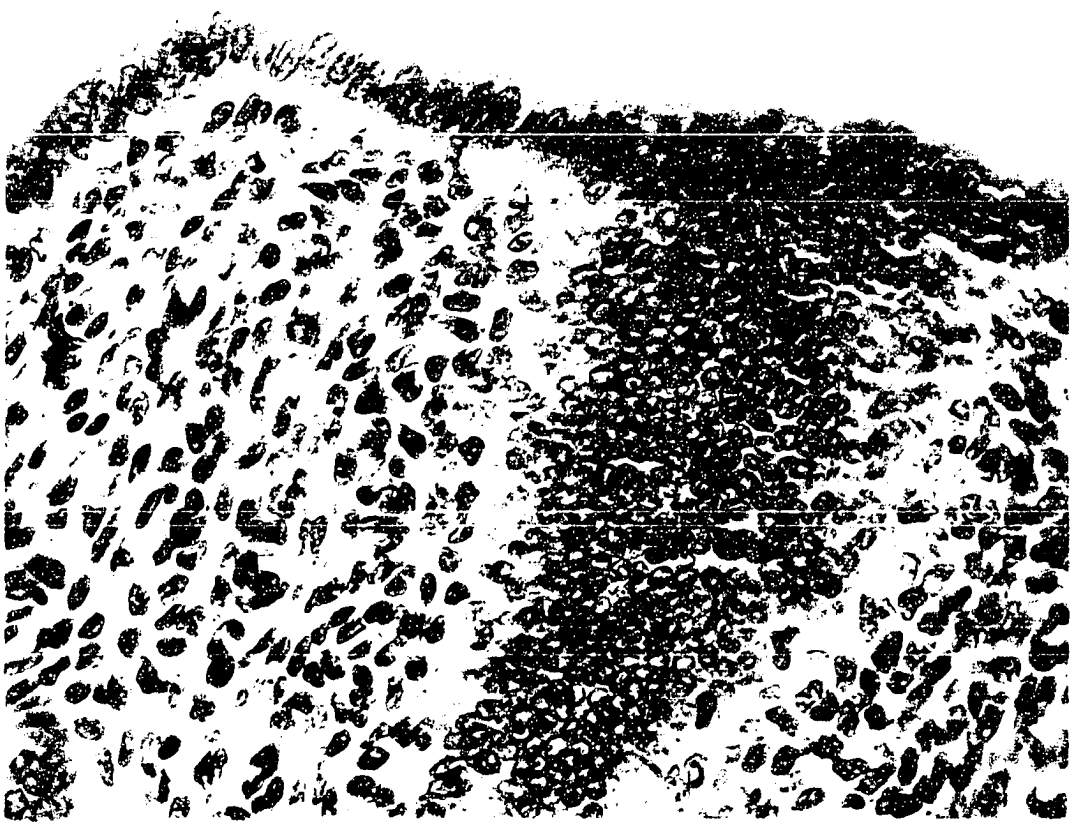

Fig. 2

PLATE II

Explanation of Figures

Figs. 3 and 4 Portion of the endometrium of intact animals showing keratinization of the uterine glands. The rats were on a vitamin A-deficient diet and autopsied during the 10th week of age.

PLATE II

Fig. 3

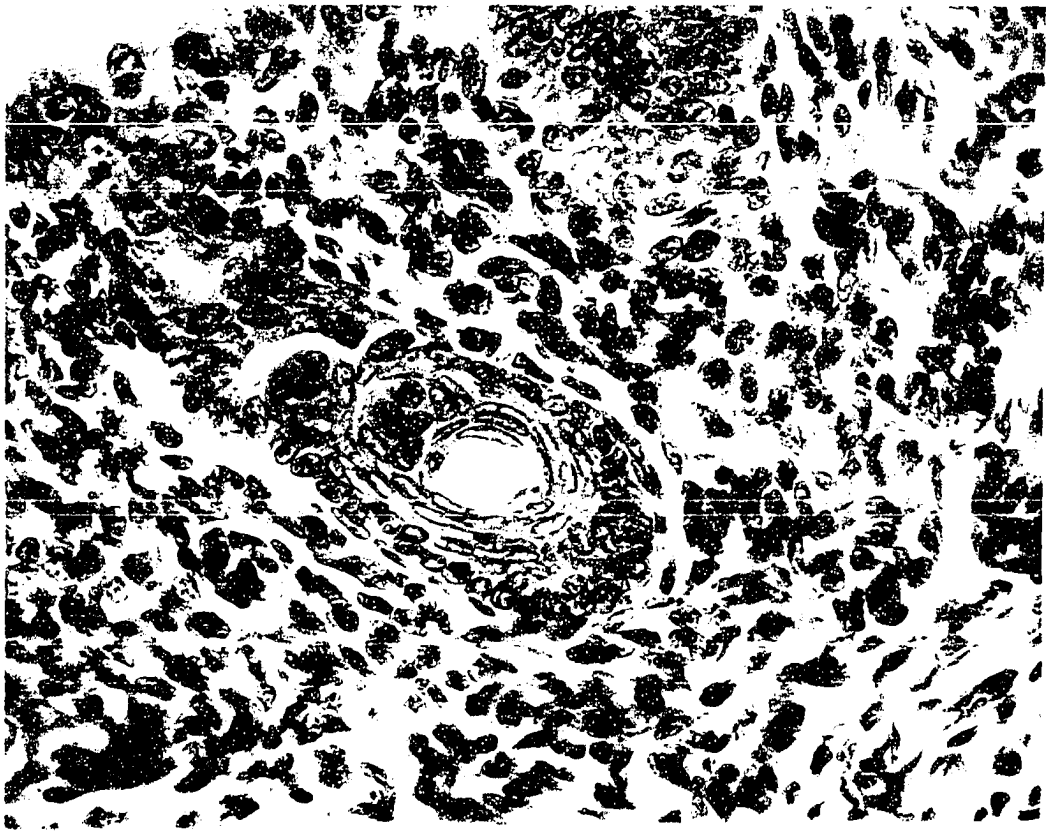

Fig. 4

PLATE III

Explanation of Figures

Figs. 5 and 6 Portion of the endometrium of intact rats showing extensive keratinization of a gland next to an apparently normal gland and normal lining epithelium. The animals were on a vitamin A-deficient diet and autopsied on the 11th and 12th weeks of age respectively.

PLATE III

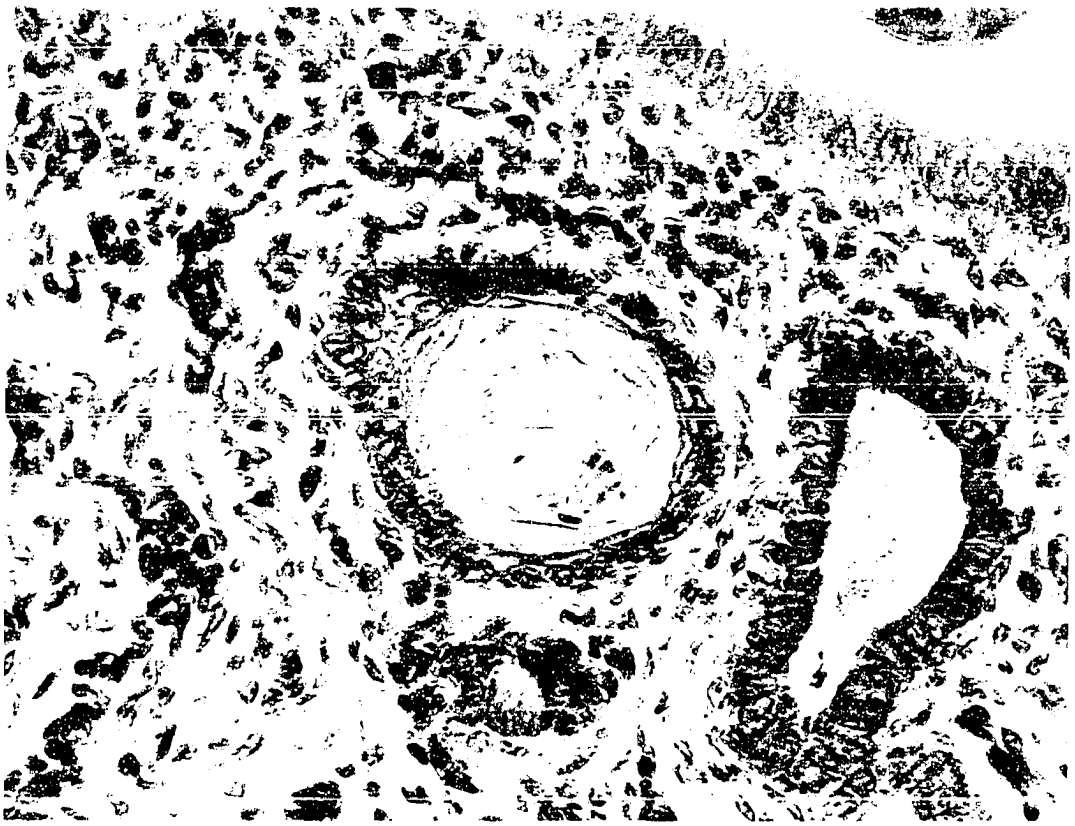

Fig. 5

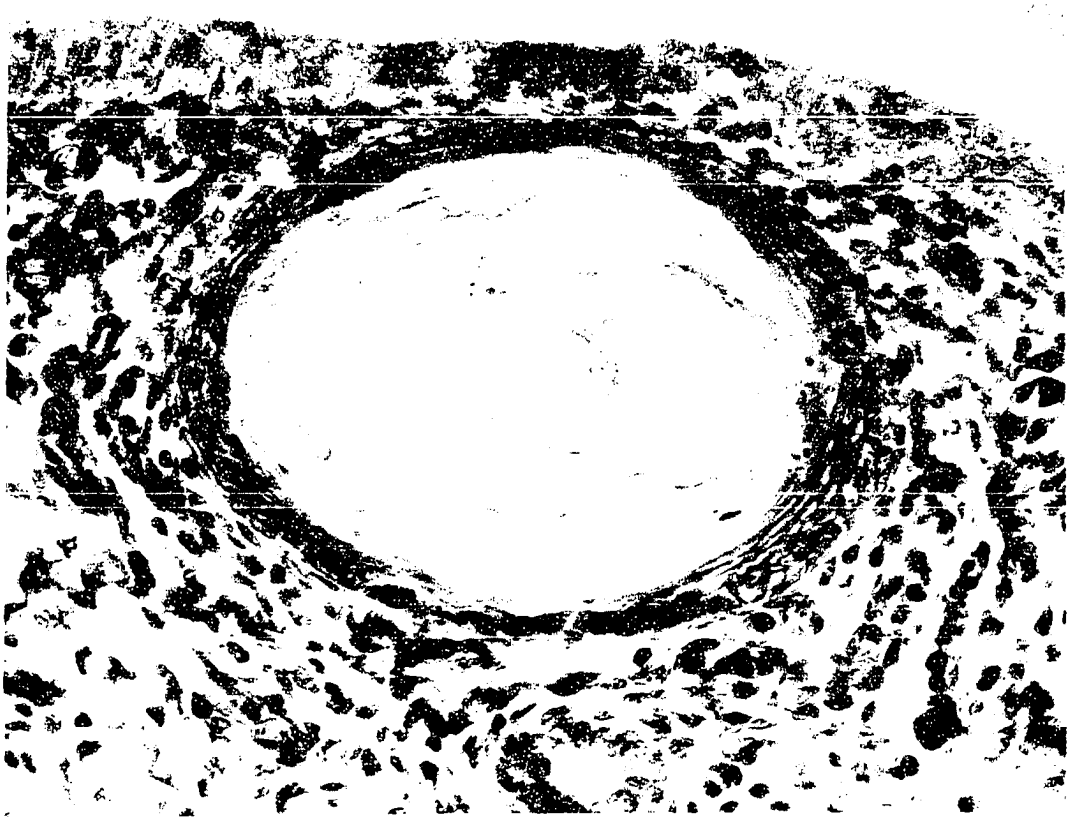

Fig. 6

PLATE IV

Explanation of Figures

Figs. 7 and 8 Portion of the uterine epithelium of ovariectomized rats. Note the absence of any metaplastic changes. The animals were on a vitamin A-deficient diet and autopsied on the 8th and 13th weeks of age respectively.

PLATE IV

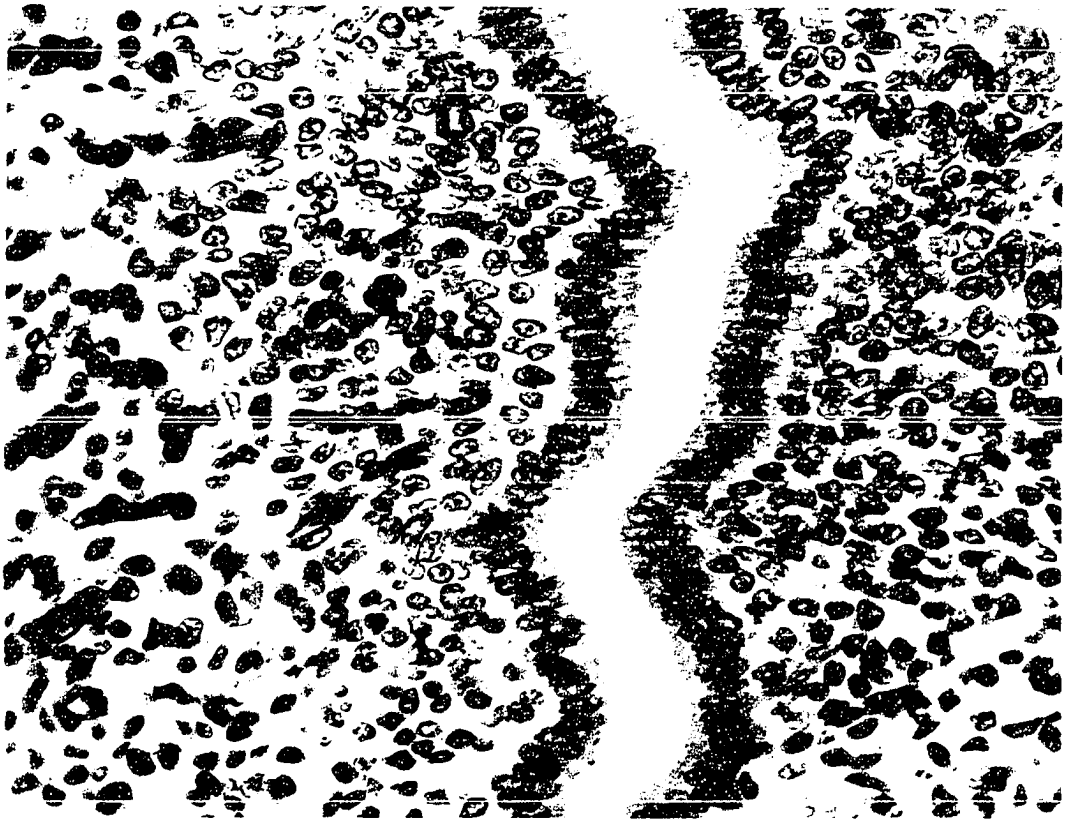

Fig. 7

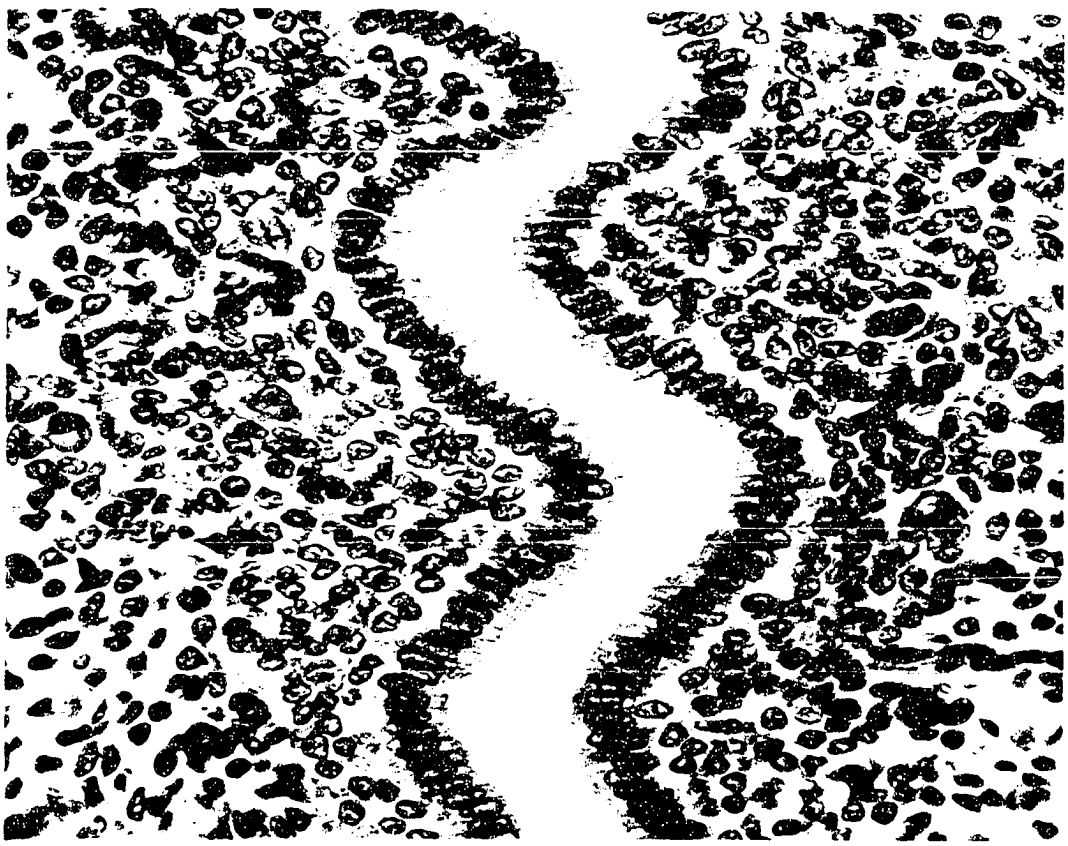

Fig. 8

PLATE V

Explanation of Figures

Fig. 9 Section of the uterus of an intact animal autopsied on the 7th day of age. Note that the uterus is not completely differentiated.

Fig. 10 Portion of the uterus of an intact rat showing stratification of the luminal cells. The animal received a total dose of 6.0 mg. estradiol dipropionate and was autopsied on the 18th day after the 1st injection. The treatment was started on the 7th day of age.

PLATE V

Fig. 9

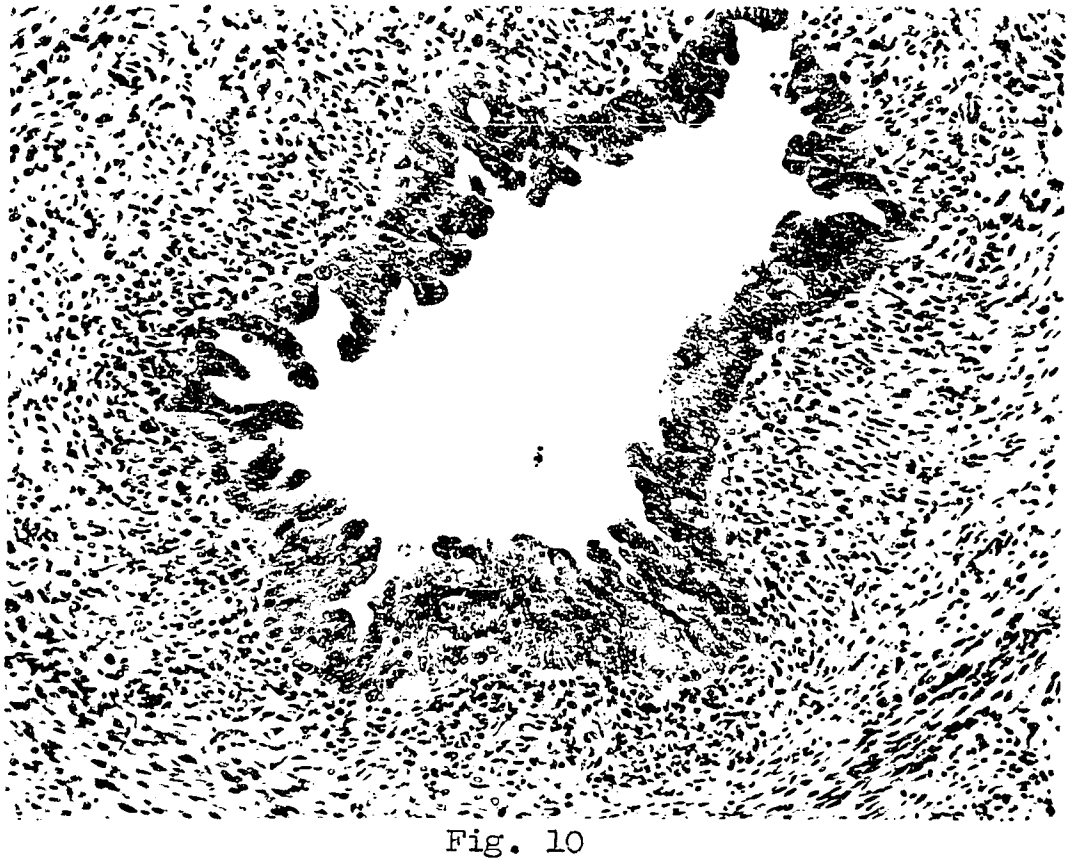

Fig. 10

PLATE VI

Explanation of Figures

Figs. 11 and 12 Portion of the uteri of intact rats showing foci of squamous metaplasia. Note the absence of an inflammatory reaction. The animals received a total dose of 8.0 mg. estradiol dipropionate and were autopsied on the 27th day after the 1st injection.

PLATE VI

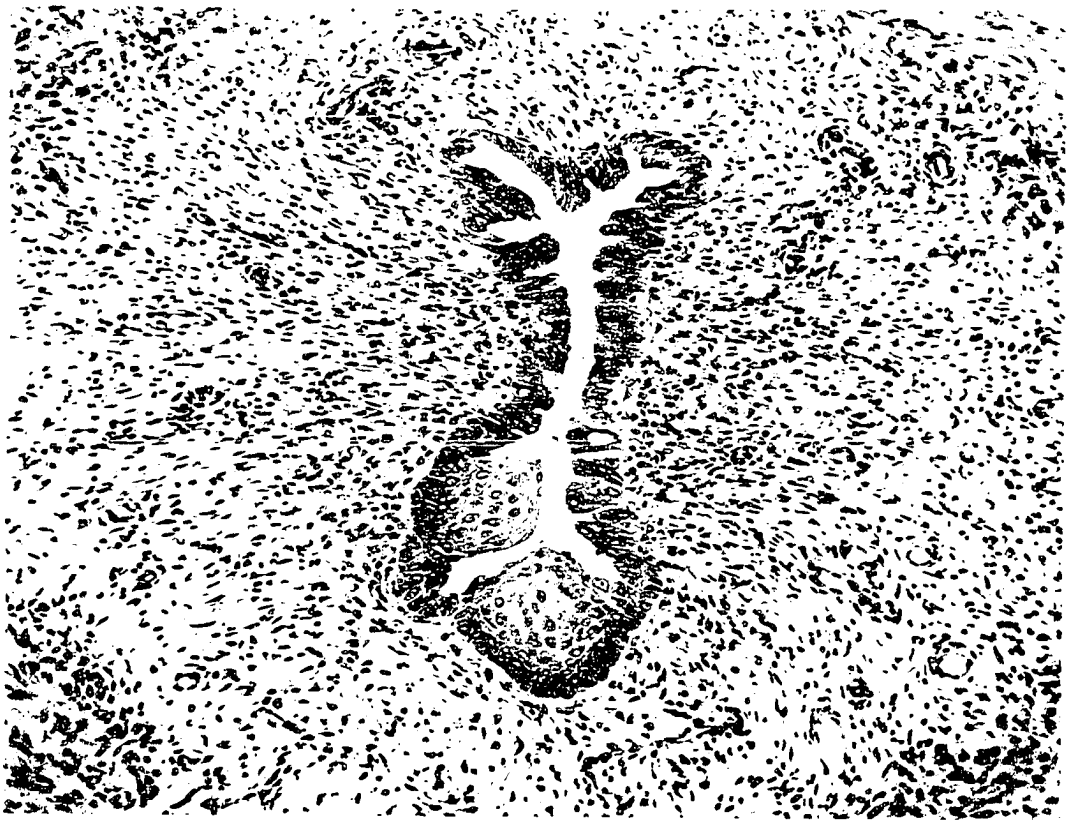

Fig. 11

Fig. 12

PLATE VII

Explanation of Figures

Fig. 13 Portion of the uterus of an intact rat showing squamous metaplasia. Note how the squamous epithelium has undermined the criginal epithelium. The animal received a total dose of 10.0 mg. estradiol dipropionate and was autopsied on the 33rd day after the 1st injection. The treatment was started on the 7th day of age.

Fig. 14 Portion of the uterus of an intact rat showing extensive squamous metaplasia. Desquamated cells are in the lumen. The animal received a total dose of 6.0 mg. estradiol dipropionate and was autopsied on the 21st day after the 1st injection. The treatment was started on the 7th day of age.

PLATE VII

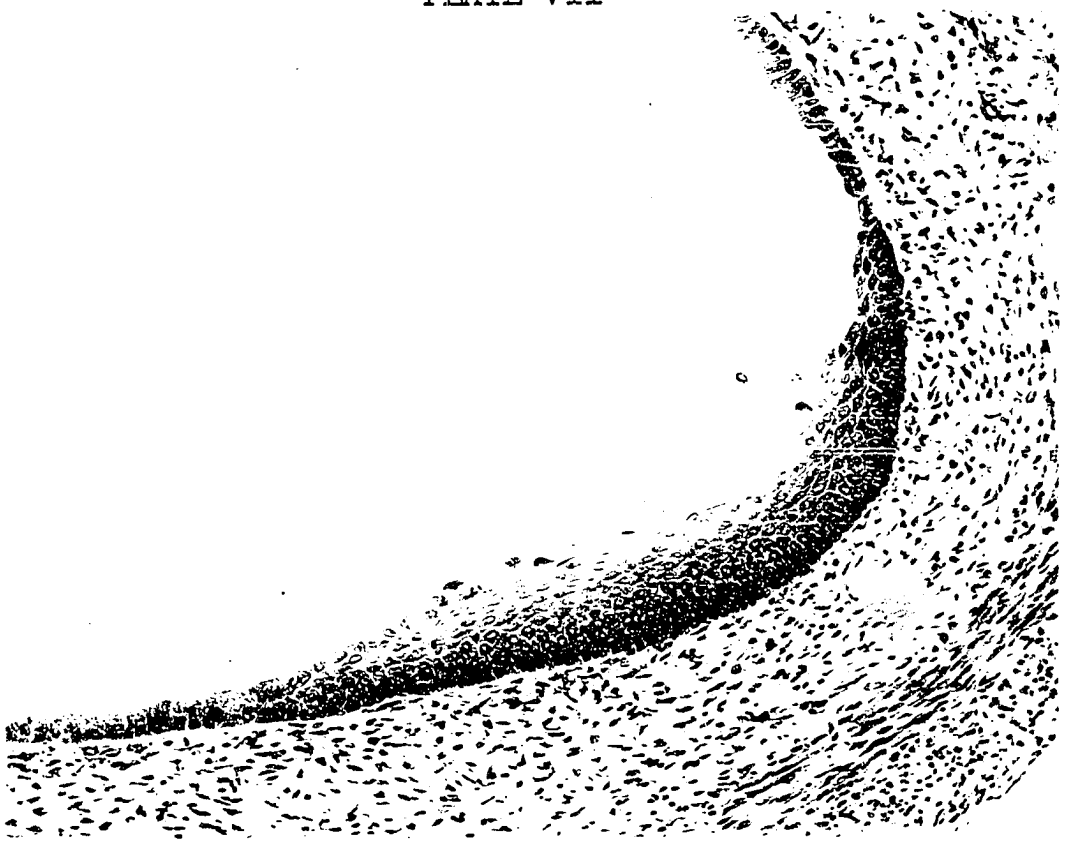

Fig. 13

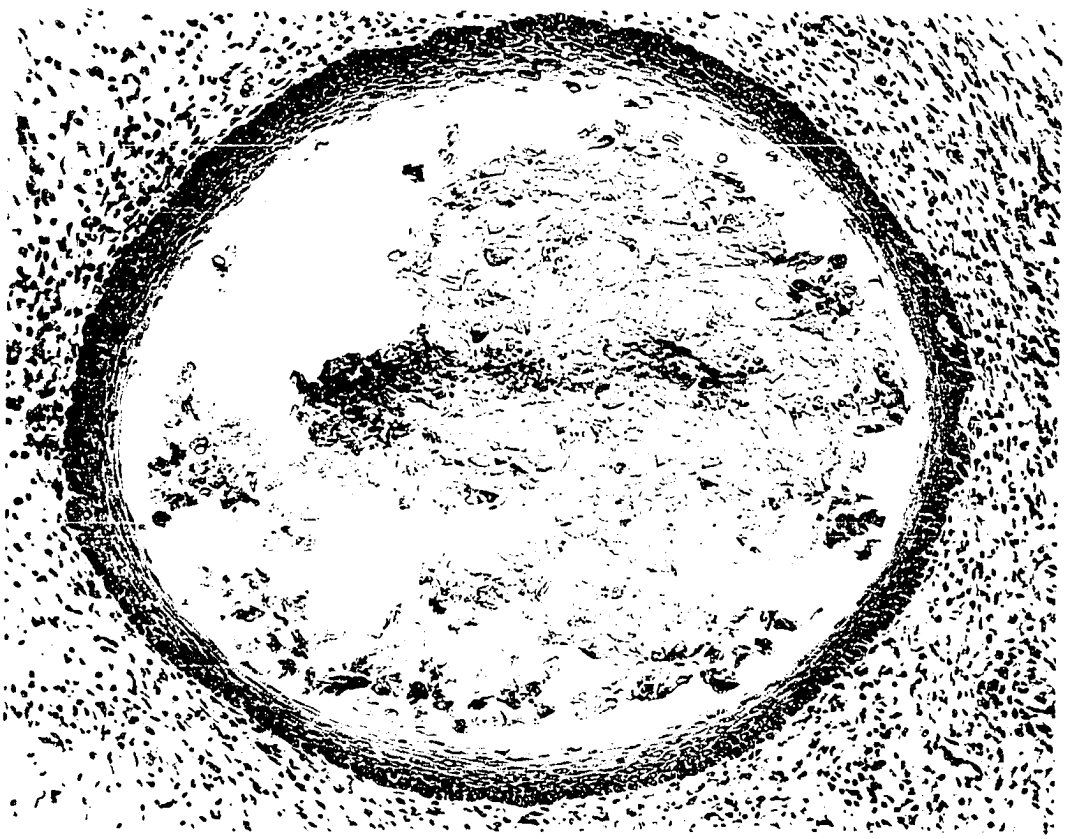

Fig. 14

PLATE VIII

Explanation of Figures

Fig. 15 Portion of the uterus of an intact rat showing extensive stratified squamous keratinizing epithelium. The lumen is filled with keratinized material, neutrophiles, and eosinophiles. The small dark staining cells in the stroma and tunica muscularis are also eosinophiles. The animal received a total dose of 8.0 mg. estradiol dipropionate and was autopsied on the 24th day after the 1st injection. The treatment was started on the 7th day of age.

Fig. 16 Portion of the uterus of an intact rat showing granulation tissue which has filled the entire lumen. Note the presence of fibroblasts, neutrophiles and blood vessels in the tissue. The animal received a total dose of 8.0 mg. estradiol dipropionate and was autopsied on the 24th day after the 1st injection. The treatment was started on the 7th day of age.

PLATE VIII

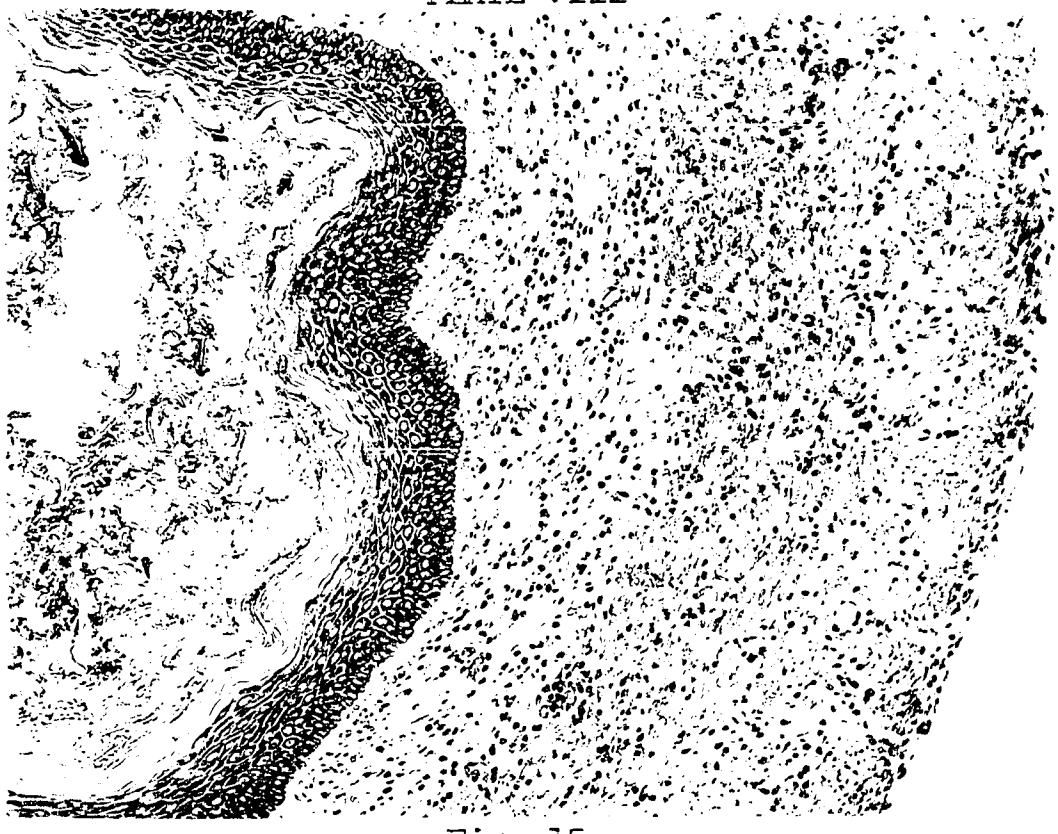

Fig. 15

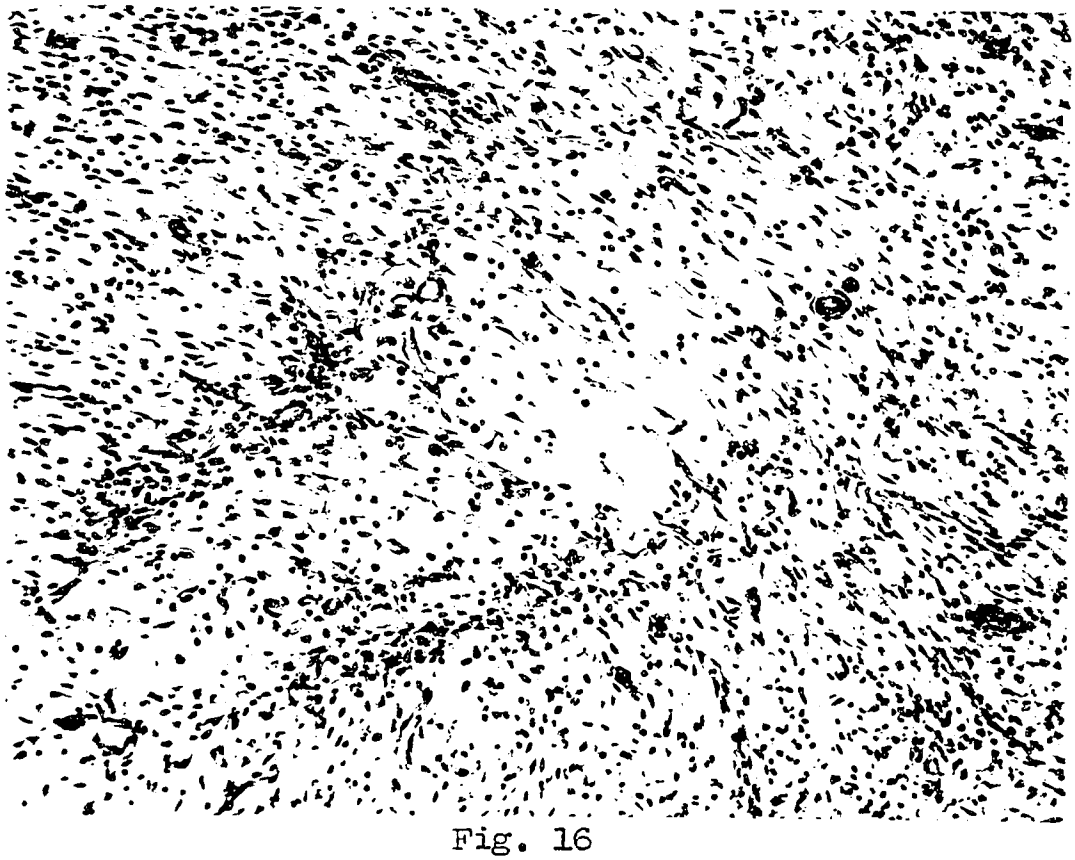

Fig. 16

PLATE IX

Explanation of Figures

Fig. 17 Portion of the uterus of an intact rat showing hypertrophy of the luminal cells. The animal received a total dose of 12.0 mg. estradiol dipropionate and was autopsied on the 39th day after the 1st injection. The treatment was started on the 21st day of age.

Fig. 18 Portion of the uterus of an untreated intact animal showing the normal structure of the lining epithelium and of the glands. The animal was autopsied on the 60th day of age.

PLATE IX

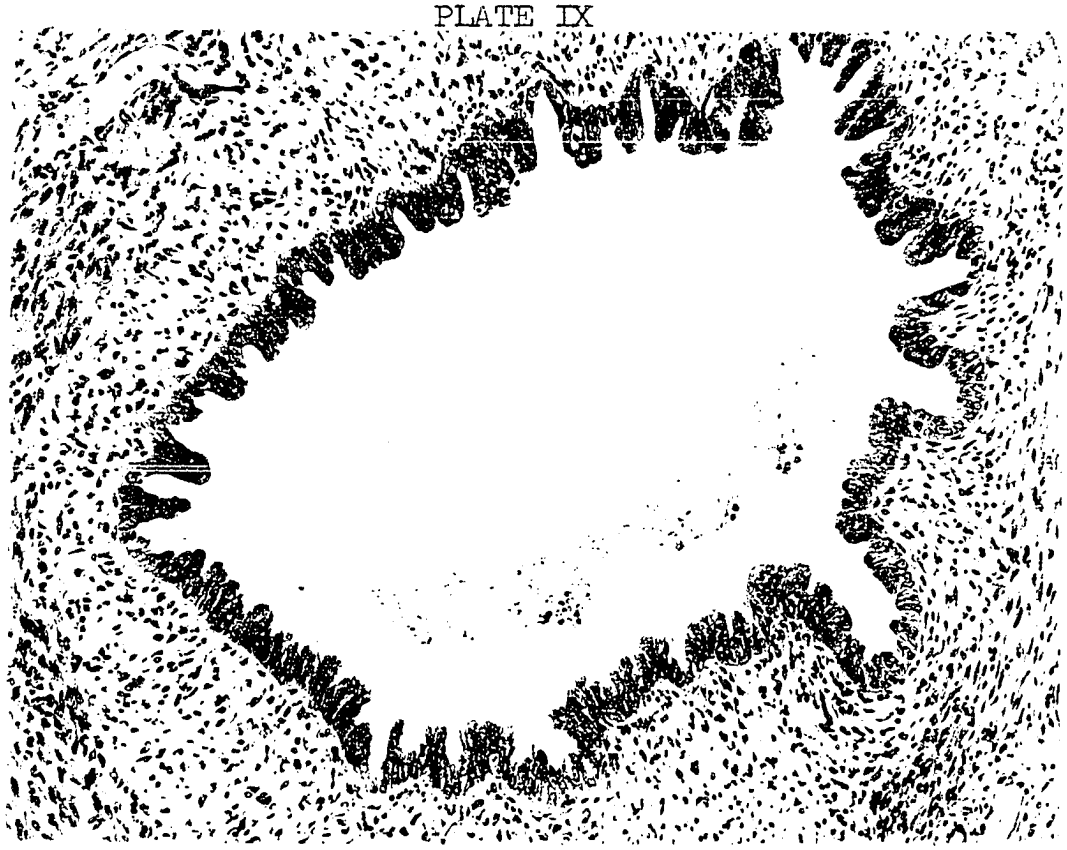

Fig. 17

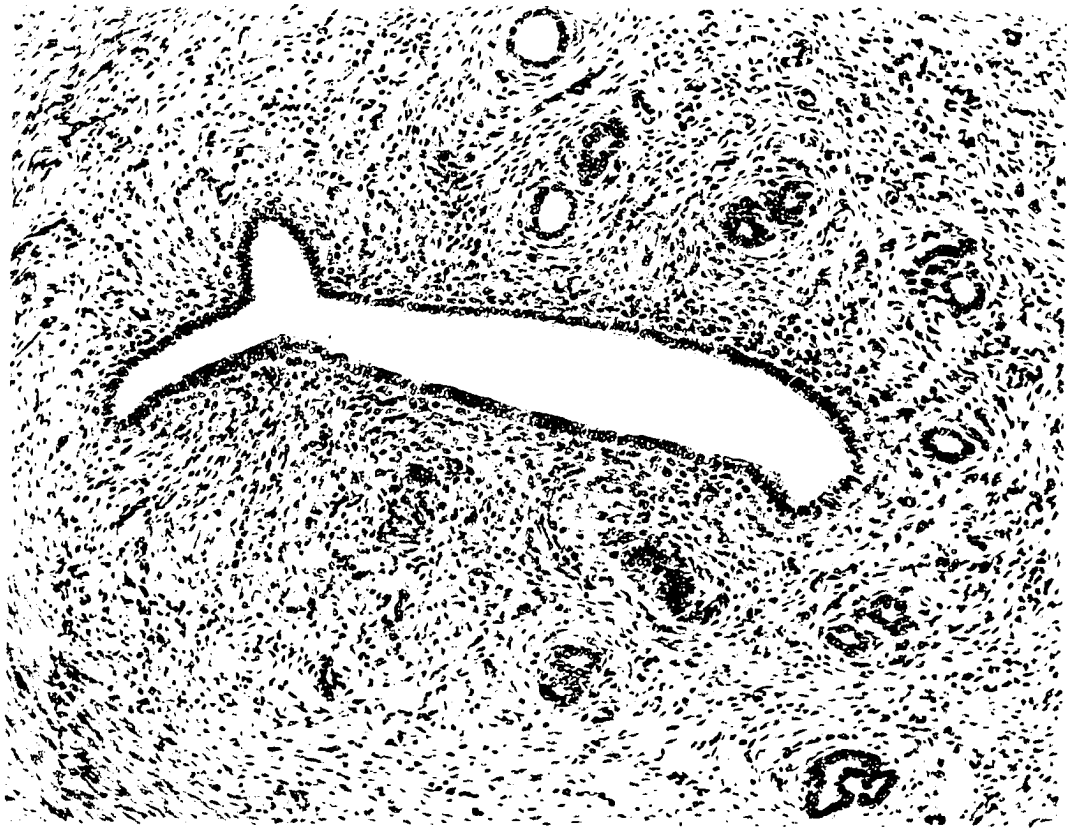

Fig. 18