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EXPERIMENTAL CARCINOMA OF THE ENDOMETRIUM

DISSERTATION

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the Degree Doctor of Philosophy in the Graduate
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By

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MAJOR FIELD OF STUDY

Pathology

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INTRODUCTION

During the studies on carcinogenesis which have been conducted in the Department of Pathology for the past 15 years, the experimental production of carcinoma of the uterine cervix was thoroughly investigated.¹⁻⁸ After trial of various procedures a technique was finally developed with which it was possible to produce carcinoma of the uterine cervix in pure bred mice of the C3H and BDF₁ strains by the insertion of strings impregnated with a saturated solution of 3-methylcholanthrene in lard. The tumors thus produced closely resembled in their gross and microscopic appearance and in their mode of development those of carcinoma in the human cervix. This success permitted investigation of some of the factors that seemed to influence tumor development. The importance of the genetic background could be clearly demonstrated, since it was not possible to develop the tumors in other laboratory animals or in the mice of other strains. It was also shown that neither the administration of estrogen nor castration had any influence on the development of the tumors.

By frequent examination of vaginal smears, the various stages of tumor development could be followed and could be verified by appropriate histological studies. These stages included initially a brief period of violent inflammatory reaction to the carcinogen, which was followed by the appearance of epithelial dysplasia, interruption of the administration of the carcinogen at this point led to a reversal of the lesions in the majority of animals. Only a small percentage continued to progress toward invasive carcinoma. Continuation of the

administration of the carcinogen soon led to the stage which was histologically and cytologically recognized as carcinoma in situ and which was followed in every instance by the development of an invasive cancer. In some animals the stage of carcinoma in situ persisted for some time, while in others it was only fleeting in appearance. The tumors thus developed invaded adjacent structures, including the rectum and the urinary bladder, and produced distinct metastases to lymph nodes, liver, kidney, and lung. This led to death of the animal through uremia or septicemia.

Electron microscopic studies of the cells during the various stages of carcinogenesis showed severe damage of the mitochondria and the endoplasmic reticulum during the stages of dysplasia and early carcinoma in situ, including the appearance of round intramitochondrial bodies whose nature remained obscure. Later, during the fully developed stage of invasive carcinoma, the cells regained cytoplasmic organelles, while the nuclear and nucleolar masses remained prominent. In advanced stages of cervical carcinoma the cells showed a series of degenerative phenomena which probably can be regarded as nonspecific effects of disturbed growth.

The results of these experiments shed light upon the precancerous stages of cervical carcinoma, specifically, the relationship between carcinoma in situ and epithelial dysplasia.

A similar study of carcinoma of the endometrium, the carcinogenesis of which in the human seems to be even more obscure than that of cervical carcinoma, was desirable. It was hoped that by such a study the significance of endometrial hyperplasia, endometrial metaplasia,

and endometrial dysplasia as precursors of the various forms of endometrial carcinoma might be investigated. The influence of the female sex hormones upon the development of carcinoma in addition to the effects of the usual chemical carcinogens might also be studied. This seemed particularly important since endometrial carcinoma in the menopausal woman definitely seems to be influenced by estrogenic substances released from ovaries or adrenals. It was particularly hoped that these experiments might clarify the important problem of the existence of a carcinoma in situ stage, which has been maintained by Halban and Hertig,⁹ but is refuted by the majority of gynecological pathologists.

In dealing with this problem of experimental carcinogenesis of the endometrium, it was felt that three different laboratory animals should be included simultaneously in this study. The rabbit was chosen since the occurrence of spontaneous endometrial carcinoma in aging animals has been reported in the literature.¹⁰ The rat and mouse were included in order to permit application of a similar technique as in the previous studies with carcinoma of the cervix. Because of the reported much more frequent occurrence of endometrial carcinoma than of spontaneous cervical carcinoma,¹¹ normal control animals were kept in much greater number and over a much longer period of observation than was the custom in our previous experiments.

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CHAPTER I
REVIEW OF THE LITERATURE

The Normal Endometrium

Rabbit.-- Since the normal ovulation of rabbits depends upon the coital stimuli, activation of the hypothalamus, and reflex release of the luteinizing hormone,¹ rabbits are classified as "reflex ovulators," in contrast to "spontaneous ovulators." Rabbits show only the follicular phase and are said to be always in a state of estrus if the nutritional condition is favorable.² The actual fluctuation of such a sustained estrus has been poorly understood since the vaginal epithelium of rabbits consists of columnar cells which do not reveal such cyclic changes as observed in the human female and in rodents, whose vaginae are lined by squamous epithelium and where marked cyclic cytologic alterations can be noted.

The genital cycle of rabbits has been studied by several authors but no definite conclusions have as yet been reached.³⁻⁶

The appearance of the rabbit uterus under light microscopy has been briefly described. In histologic sections the uterine cavity appears stellate on cut surface; there are six endometrial folds, two of which are on the mesometrial side and transform later to placenta.² During estrus the surface layer of epithelial cells is straight and almost continuous, being rarely broken by short glandular invaginations.² Klein⁷ described two types of endometrial epithelial cells--large cuboidal cells which were frequently ciliated cells, and high columnar cells with fusiform nuclei. Marshall⁸ found little evidence of

activity in the uterine glands during the heat period. Hamilton⁵ said that the anterior regions of the uterine horns are lined by tall ciliated columnar cells and the posterior regions by nonciliated cells. In the latter regions the epithelium is less crowded and more round nuclei are present. The glandular epithelium throughout the uterus is formed by short nonciliated cells.

Larsen⁹ described the castrated uteri as reduced in size, the lumina less complicated in structure, and the epithelium considerably lower with fewer glands and shallower extension. Ciliated cells had disappeared. Cytoplasmic basophilia was reduced. Methylene blue metachromatic granules were absent, and PAS-positive substance was noted only in the basement membrane. The cell surfaces and the luminal cytoplasm were PAS-positive. Both ciliated and nonciliated cells were seen during the estrous phase. The nonciliated cells were more numerous and had narrower, redder cytoplasm. The glandular epithelial cells resembled the nonciliated cells. PAS-positive substance was found on the luminal surface and in the cytoplasm of nonciliated cells but was absent in glandular or ciliated cells. In pseudopregnancy multinucleated cells were found.

The electron microscopic structure of the rabbit endometrium has been studied by Larsen.⁹ In castrated rabbits the cells seemed poorly developed. The usual cytoplasmic vacuoles and granules were not recognized, and microvilli were reduced in number. Mitochondria were also fewer. Electron-dense bodies were still present, and only small vacuoles and Golgi apparatus were seen.

During estrus, nonciliated cells were taller with ovoid nuclei

and with many microvilli on the luminal surface. The lateral cell surfaces were slightly undulating, and many desmosomes were seen. In the cytoplasm near the lumen were large vacuoles containing filiform or granular material, and there were some communications between these vacuoles and the cell surface. Numerous inclusions which varied in size and density were particularly abundant in the mouths of the glands and less numerous in the depths of the glands. Mitochondria were numerous and contained one or two electron-dense bodies. Adjacent to the nucleus the Golgi apparatus was seen. The ciliated cells were less electron-dense. Their nuclei were less irregular than those of the nonciliated cells. The mitochondria were smaller and contained electron-dense bodies. Both microvilli and cilia were present. The cilia contained two central and nine peripheral filaments. No specific cytoplasmic inclusions were seen. The endoplasmic reticulum was poorly developed. The Golgi apparatus was always present.

Rats.--During the diestrus the uterus measures 1.7 mm. in diameter, is slender, and has a slit-like lumen. It is lined by simple columnar epithelium with a delicate cuticular membrane adjacent to the lumen. In the normal postestrus stage the surface epithelium for one to three days is in the process of rapid and complete repair with many mitoses present (one to two per oil immersion field).¹¹ The surface epithelium consists of low cuboidal cells 15-18 μ in height. A large oval nucleus occupies most of the cell. The glandular spaces are nearly collapsed and the secretion seems to have disappeared. In the normal diestrus the leukocytic infiltration persists, but in pseudopregnancy it is negligible and lasts only five to six days.

The uterus becomes distended during proestrus due to the accumulation of watery, noncoagulable fluid in the lumen, preceded by hyperemia. The columnar epithelium now appears cuboidal and secretes a large amount of mucus. The endometrial stroma and the myometrium are extensively infiltrated by leukocytes, particularly eosinophils.

During the estrous phase the uterus is at its largest and shows marked hyperemia. During the late estrus the fluid is suddenly released and the lumen collapses. The epithelium becomes columnar and vacuolic degeneration begins. The glandular spaces do not collapse until two days later.

During the early metestrus the epithelium of the endometrium shows vacuolic degeneration and dehiscence, with some leukocytic infiltration. After the peak of vacuolic degeneration which started in the previous stage has passed, regeneration begins.

The ultrastructure of the rat endometrium has been described briefly by Berlingieri and his associates.¹² During estrus, the uterine epithelium consisted of a single layer of tall cells. The cytoplasm appeared homogeneous or had an areolar appearance and was rich in organelles. Microvilli were numerous and long. The subluminal portion of the cytoplasm showed many spherical vacuoles 0.1-0.3 μ in diameter; some of them contained dense granulations. Mitochondria were abundant and had the usual appearance in the compact cells but were swollen in the areolar cells. The ergastoplasm was regular and well-developed. In the supranuclear region the Golgi apparatus was observed, which in the areolar cells was large with an irregular contour and very prominent vesicles; in the compact cells the vesicles were less prominent.

During diestrus, the cells were of low cuboidal type. The free borders of the cells were without microvilli, and the subapical vacuolic layer had disappeared. Mitochondria were swollen or small and dense, and the Golgi apparatus showed only a few vesicles although the ergastoplasm was well-developed. Lipid inclusions were abundant and some had a lamellar structure.

The endometrial cells of castrated animals were much shorter and contained less cytoplasm. The cell surface was flat; vesicles were decreased but lipids were increased. Mitochondria were reduced and appeared dense, ergastoplasm was rudimentary, while the Golgi apparatus was similar to that during the diestrus. Desmosomes were reduced in number.

Mouse.--The estrous cycle of the mouse has been studied extensively.^{13,14} During diestrus the endometrium consists of 4-8 layers of poorly stained cells which apparently form a syncytial mass. In the surface epithelium only occasional mitoses are seen. Heavy leukocytic infiltration is noted. As in early proestrus, mitoses are most frequent in the glandular epithelium.

During proestrus, the luminal fluid is increased in amount but contains no cells. The surface epithelium is made up of low columnar cells with a distinct basement membrane, and mitoses are frequent. No leukocytes are seen within the epithelium and only few are noted in the subepithelial zone. The glandular lumen is distended, and the glandular epithelium shows many mitoses. The stroma seems very cellular.

During estrus, the endometrial cavity contains small masses of

free cells. No basement membranes can be recognized, and the surface cells are pseudostratified with distinct lateral borders. The cytoplasm stains poorly. Almost no mitoses are present. The subepithelial regions are heavily infiltrated with leukocytes. The glandular epithelium shows only minimal secretory activity. The lumina of the glands are slightly distended, and a few mitoses and leukocytes are present.

The electron microscopic structure of the endometrium of the laboratory mouse has been extensively studied by Nilsson.¹⁵⁻²⁰ In the spayed animal the surface epithelial cells of the endometrium measured 7-10 μ in height. The luminal cell surfaces contained microvilli measuring 0.2 μ in maximal length. The cytoplasm contained small rod-like mitochondria with a few dark granules. The endoplasmic reticulum was poorly developed. The Golgi apparatus was at the upper pole of the nucleus. There were a few granules, and only a few vesicles with granules were present. The following cytoplasmic inclusions could be distinguished: (1) vesicles with ring-shaped profiles bordered by single membranes; (2) slightly dense ovoid granules with smooth outlines, containing amorphous substance of varying electron-density; (3) dark ovoid homogeneous granules with smooth outlines occasionally surrounded by the membrane; (4) dark homogeneous granules having an irregular outline and no border membrane--these were the most frequent; (5) small vacuoles with membranes, located near the luminal surface; (6) granular bodies 0.5 μ in diameter.

During the normal estrus of the mouse the surface cells measured 18-22 μ in height. The luminal surface had microvilli 0.6-0.7 μ in

length in which occasional vacuolar spaces might be seen. The lateral cell surfaces showed parallel folding. Occasionally intercellular vacuoles were noted. Mitochondria appeared as blunt rods, and some of them contained spherical homogeneous bodies surrounded by a single membrane and having a maximal diameter of $0.15\ \mu$. The endoplasmic reticulum was increased, and increased ribosome granules appeared in bundles. The Golgi apparatus was extensively developed. Cytoplasmic inclusions were similar to those present in the castrated animals.

When short-acting estrogen was administered to spayed mice, a change was noted as early as four hours after treatment. The cell height was slightly increased, so was the length of the microvilli. There were also bleb-like protrusions of the luminal cell surface. Several different round bodies with coarse granules and/or membranes were found. Dark granules, vesicles, and vacuoles as seen in the spayed animals were present. Twenty hours after therapy the cells were taller. The endoplasmic reticulum was well-developed and elongated, the Golgi apparatus showed large vacuoles, dark ovoid granules were more numerous, and the structures with round profiles were seen most frequently. After two days the cell height was the highest, and the microvilli were shorter. The luminal substance seen earlier decreased and then disappeared. On the fourth day the cells measured $10-12\ \mu$ and had short microvilli. Mitochondria were at their largest size. After the sixth day no difference could be detected between the spayed and the treated animals.

When spayed mice were observed after a single injection of long-acting estrogen, the cells were $18\ \mu$ in height within three days. The

microvilli reached the maximal length of 0.8μ . Nuclei were basally located. The mitochondria were spherical and included some dense homogeneous bodies 0.1μ in diameter, usually surrounded by a membrane. The endoplasmic reticulum was elongated and varied in size. In the Golgi region many vesicles and vacuoles were present. Cytoplasmic inclusions were increased in the apical cytoplasm. Near the luminal surface, vesicles were present with opaque contents or vacuoles. Large dense vesicles were limited to an area beneath the luminal surface. Lipid granules were practically absent.

According to Rhodin,²² in the glandular epithelium of the mouse endometrium the cytoplasm beneath the luminal surface was rich in secretory granules showing all gradations of density. Ribosomes were abundant. Rough-surfaced endoplasmic reticulum and mitochondria were scarce in the apical cytoplasm. The basal cytoplasm showed abundant rough-surfaced endoplasmic reticulum and ribosomes. Large accumulations of lipid granules were seen near the basement membrane.

Spontaneous Endometrial Carcinoma

Rabbits.--The incidence of spontaneous endometrial adenocarcinoma in rabbits first reported in 1900 has been extensively investigated. Its incidence has been reviewed in recent years.^{23,24} By 1940 there had been many case reports on the spontaneous endometrial carcinoma of rabbits.²⁵⁻⁴⁴ The first of the extensive and systematic studies Greene⁴⁵⁻⁴⁹ has made in this field was reported in 1938. Over a nine-year period he observed a colony of 849 rabbits representing 14 different breeds, in which more than 142 adenomas and adenocarcinomas

developed. No tumor appeared in an animal under two years of age.

The age distribution was as follows:

<u>Age when tumor appeared</u>	<u>No. of rabbits</u>	<u>No. with tumors</u>
2-3 years	491	21
3-4 years	259	54
4-5 years	71	45
5-6 years	24	19
6-7 years	<u>4</u>	<u>3</u>
Total	849	142

Only Belgian and Rex breeds were free of tumors. The frequency of spontaneous occurrence of tumor was in the following order: Tan (50%) French Silver (25%) Havana (22%) Dutch (20%) Martan (17%) English (17%) Chinchilla (17%) Beveran (15%) Sable (15%) Himalayan (10%) Polish (8%). Among 148 progeny of a tumor-bearing animal, 34 developed tumors. Some transmissible physical variations were also genetically related to the malignant disease, i.e., dwarfism⁵⁰ in Dutch rabbits. A second constitutional abnormality was "cretinoid change." Toxemia of pregnancy^{51,52} was another factor considered. Histological study of the rabbits which developed toxemia revealed involvement of the liver, adrenal, and kidneys. Hyperestrinemia after the liver injury might be considered as one of the etiologic factors.

Fifty-three rabbits with uterine cancer had tumors of other organs.

The uterine tumors at the time of detection were the size of ten-day pregnancy cysts and in six months reached the size of a hen's egg. Frequently they were bilateral. Often the metastasis coincided with

decrease in size of the primary tumors. The animals died 50-20 months (average 12 months) after the tumor was first detected clinically. Metastasis developed later than one year after the tumor made its appearance.

Burrows^{53,54} regarded all 15 uterine carcinomas found among 25 rabbits in his experiment as spontaneous.

Dobberstein and Tamaschke⁵⁵ described an endometrial carcinoma which they found in a four-year-old Angora rabbit.

Lombard⁵⁶ found three carcinomas in animals aged about two years in a commercial colony of some 500 white Angora rabbits.

When commencing their experiments, Merriam et al.⁵⁷ found two spontaneous adenocarcinomas in the endometrium of adult rabbits.

Ingalls et al.⁵⁸ reviewed the records of 1,735 female rabbits in the Phipps Colony during a 30-year period and found uterine tumors in 353, or 24.4 per cent, of the animals. The percentage varied from 8.8 per cent in a group aged 2½-3 years to 58.9 per cent in a group over four years of age. The incidence was higher among breeders than non-breeders. The animals having high degree of resistance to tuberculosis had a lower incidence of tumors.

Rats.--Bullock and Curtis^{59,60} observed four adenocarcinomas of the uterus among 31,868 rats. The animals ranged between 13 and 30 months in age (average 20 months). One adenoacanthoma was observed in a rat aged 20 months. Other uterine tumors found in the total of 334 tumor-bearing animals were 19 sarcomas, three carcinosarcomas, and ten squamous carcinomas. The adenocarcinomas and adenoacanthoma were found

in the Fischer, August and Marshall strains and a hybrid between Fischer and August.⁶¹

In 1934 Guérin and Guérin⁶² reported the occurrence of an atypical carcinoma of the uterus in a white rat which they were able to transplant. An electron microscopic study of the Guérin tumor⁶³ showed the presence of intercellular particles resembling viruses.

Ratcliff⁶⁴ reported finding five adenocarcinomas of the uterus among 295 female rats of Experimental Colony strain whose average age was 780 days.

In his publication, Guérin⁶⁵ mentioned 18 uterine tumors of rats; 13 of them were carcinomas, two sarcomas, and three mixed tumors. In addition 27 benign tumors of the uterus were mentioned.

Crain⁶⁶ found three adenocarcinomas and seven sarcomas of the uterus among 786 albino rats.

Gilbert and co-workers^{67,68} reported the tumor occurrence in three substrains of the Wistar rats. Ten uterine adenocarcinomas were found all in GG-strain females (total female 552).

In carcinogenic experiments with plastics, Hueper^{69,70} noted nine adenocarcinomas of the endometrium in a control group of Bethesda Black rats. One carcinoma was found among 29 rats ranging from 19 to 21 months of age, and eight carcinomas were found among 82 rats 22-24 months old.

Nesswetha and Meiners⁷¹ found an adenocarcinoma of the uterus in two rats of Wistar strain.

Morris et al.⁷² found an adenocarcinoma of the uterus among the 36 female rats. The tumors were found at 18 months. Morris and

co-workers⁷³ also found one adenocarcinoma of the endometrium among the 17 Buffalo rats. The animal was 20 months old.

Schulze⁷⁴ reported a high incidence of uterine adenocarcinoma in rats. Of the 114 Bethesda Black rats 16 had carcinomas of the uterus. The age range of most of the tumor-bearing animals was 601 to 1000 days. One uterine sarcoma was also present. Among the 690 rats of Sprague-Dawley strain, seven had uterine carcinomas; four uterine sarcomas were also found. Like Gilbert, Schulze was impressed with the high incidence of tumors arising in the endocrine glands.

Snell⁷⁵ noted seven tumors of the uterus: one adenocarcinoma, one squamous cell carcinoma, and one sarcoma from among 23 Buffalo rats; one and two angiosarcomas among 20 M520 and 23 F344 (Fischer) rats, respectively; one adenocarcinoma among 29 AC I(AxC) rats. All rats were over 18 months old.

Singer⁷⁶ obtained a spontaneous uterine sarcoma from a rat. Pollis⁷⁷ found a uterine sarcoma in an albino rat. Thompson et al.⁷⁸ found two leiomyosarcomas of the uterus among 82 Sprague-Dawley rats.

Recently Kobayashi and his associates⁷⁹ discovered two spontaneous adenocarcinomas of the uterus in non-inbred albino Japanese rats 7-11 months old. Their electron-microscopic studies showed the presence of the intercellular lamellar structure. The growth of the transplanted tumors was enhanced by the administration of estrogens and suppressed by androgens.

Mouse.--The occurrence of spontaneous endometrial carcinoma in mice is very rare.^{81,82} Slye, Holmes and Wells⁸⁰ in their observation of 39,000 mice found seven sarcomas, three benign adenomas, and one

teratoma of the uterus. Slye⁸³ and Slye and co-workers⁸⁴ reported one squamous carcinoma of the uterus and four sarcomas in their study of spontaneous tumors in mice, and Woglom⁸⁵ described one carcinoma of the uterus with liver metastasis which he found in a mouse. Heidenhain⁸⁶ has described two spontaneous carcinomas which he found in white mice at least 25 and 24 months old.

Snell⁸⁷ reported two hybrid mice with carcinoma simplex at the Jackson Memorial Laboratory.

Gardner and Pan⁸⁸ found 13 spontaneous tumors arising in the vagina, cervix, or uterus among 56 female mice of PM stock; four undifferentiated tumors and one spindle cell sarcoma arose in the uterine horns.

Fischer and Khl⁸⁹ mentioned two uterine corpus carcinomas in their publication. Mouriquand and Mouriquand⁹⁰ found in the first ten generations of PS strain mice a total of 736 tumor carriers out of 830 animals examined. One adenocarcinoma and one leiomyosarcoma of the uterus were found.

Green⁹¹ stated that they observed 10-15 spontaneous endometrial carcinomas in mice, mainly in the BALB/cAn substrain. The tumor carriers were 18-30 months old. These tumors were transplantable in the BALB/c and C3H/HeN strains.

Strong⁹² found three sarcomas in CBA mice. Gurin⁶⁵ recorded three uterine sarcomas. Biancifiori and Ribacchi⁹³ found nine sarcomas and one leiomyoma among 100 virgin CBA/Cb/Se (female) x BALB/cf/Cb/Se (male) F₁ hybrid mice. Gardner⁹⁴ mentioned frequent occurrence of uterine fibrosarcomas in some strains of mice.

The Carcinogenic Effect of Estrogens upon the Endometrium

Rabbits.--In general, more time is required for the production of carcinoma in rabbits than in mice and rats, and rabbits are under poorer genetic control. Few experiments on the carcinogenic effect of prolonged estrogen administration on the endometrium of the rabbit have, therefore, been made, although many reports of short-term pharmacological studies have appeared in the literature. Furthermore, since the high incidence of spontaneous endometrial carcinoma in these animals is now recognized, the true significance of positive results is under question.

Borst,⁹⁵ using progesterone and estrone, produced an adenomatous change in castrated rabbits. This finding was later reported by Pierson⁹⁶ in more detail. One rabbit, which had received over a period of ten months 104 injections of 0.1 mg. of estrone, showed a 5 x 4 cm. papillary adenoma in one uterine horn. Another rabbit, which received 104 injections of 1.01 mg., showed rather diffuse malignant adenomatous change of the uterus with squamous metaplasia and invasion of the underlying myometrium to the perimetrium.

Lacassagne⁹⁷ treated six female newborn rabbits with weekly injections of estrone benzoate in oil. For the first two months the injections were of 1,000 I.U., after which they were increased to 10,000 I.U. per injection. One rabbit kept for 28 months showed pronounced epithelial change in the endometrium with invagination and infiltration reaching the serosa.

Zondek⁹⁸ reported negative results with estrone and estradiol.

Pierson⁹⁹ treated 16 castrated rabbits every day with Prolan. Two out of ten rabbits treated for one year developed polypous changes in the endometrium, one of which showed infiltration of the myometrium and cellular atypia. One of three rabbits treated for 7½ months developed an adenoma which had infiltrated the myometrium.

In reports of her later experiments, Pierson^{100,101} described the development of cystic papillary adenomas with atypical glands and infiltrating nests of squamous cells in the endometria of four rabbits treated twice weekly with 0.1 mg. of estrone for 13-21 months. The squamous cells were undifferentiated and showed nuclear irregularity. In the nests of squamous cells occasional hyalinized cartilaginous changes and osteoid formations were noted. Pierson¹⁰² also found an atypical adenoma in one of three rabbits which had received two implantations of bovine anterior pituitary 18 months previously.

Sasaki¹⁰³ subjected 38 castrated rabbits to oral administration of o-aminazotoluene and 500 m.u. of estrone benzoate or estrogen-progesterone mixture. One of the four rabbits that survived for over one year developed an atypical adenoma.

Paladino¹⁰⁴ injected 4,4'-dipropionate-alpha, beta-diethylstilbene into a castrated female rabbit. An adenomatous formation with infiltration of the underlying myometrium was noted on one rabbit after receiving 0.03 mg. daily, or a total of 4.74 mg.

Chevrel-Bodin and Cormier¹⁰⁵ reported negative results with estrone.

Meissner et al.^{106,107} studied the influence of alloxan-diabetes and estrogen administration in the development of endometrial carcinoma in female virgin rabbits. The 48 rabbits used were divided into four

groups and treated as follows: (1) seven animals were given diethylstilbestrol dipalmitate alone in varying amounts; (2) 11 animals were given alloxan and stilbestrol; (3) 20 rabbits received alloxan alone; (4) ten rabbits served as control. The six carcinomas that developed occurred only in the animals receiving stilbestrol--two of them in the first group, which received stilbestrol alone, four in the group receiving alloxan and stilbestrol. In the first group, the animals developing carcinoma received 70 mg. of diethylstilbestrol dipalmitate in 14 doses; they died at 315 and 389 days. In the group of alloxan-diabetic animals, one animal developing carcinoma had received 65 mg. of diethylstilbestrol dipalmitate for ten months; the second, 45 mg. for two weeks; the third, 70 mg. for eight months; and the last, 75 mg. for ten months. Each dose was of 5 mg. The animals died or were killed at 339, 413, 435, and 616 days of the experiment. Histologically, two of the tumors were adenocarcinomas combined with adenomatous hyperplasia of the endometrium, one was a well-differentiated multifocal adenocarcinoma, one an adeno-acanthoma, one a carcinoma simplex, and one an undifferentiated carcinoma. Endometrial hyperplasia occurred after 89 days, and villous proliferation of the endometrium was noted after 600 days. Pyometra was also noted.

Rats.--Halter,¹⁰⁸ using 30 female white rats of Wistar stock, by daily injections of sheep anterior pituitary extract, obtained an adenocarcinoma of the endometrium. This animal received 123 injections in a daily dose of 0.5 ml. At 104 days the midsection of the left horn revealed a nodule 8 mm. long. At necropsy at 256 days, it was noted

that the tumor had also involved the ureters, ovaries, mediastinum, lungs, peritoneum, and omentum.

McEuen¹⁰⁹ found one squamous carcinoma of the uterine horn in the group of rats serving as controls for his experiment using estrone; he presumed this to be the result of contamination of the animal. In a later report,¹¹⁰ he mentioned two rats (one castrated, one non-castrated) having squamous carcinomas of the uterine horns following the administration of estrone.

Pfeiffer,¹¹¹ using 185 newborn female rats, transplanted to them the testis of a littermate within a few hours of birth. One adenocarcinoma of the endometrium was found after 816 days.

Prigodzina¹¹² produced an adenocarcinoma of the uterus in 11 out of 103 castrated white rats treated with Synestrol.^(R) The estrogen was applied subcutaneously once monthly for 4-6 months in a 30 per cent solution in 10 mg. of wax. The tumors started after two months of treatment.

With diethylstilbestrol, Dunning et al.¹¹³ produced one adenocarcinoma of the uterus. Their experiments used a total of 224 rats of several strains. The uterine carcinoma occurred in the hybrid strain Cx(AxC). Thirty female rats, beginning at 108 days of age, were given an average of 5.4 mg. of stilbestrol in cholesterol pellets implanted in the subcutaneous tissue. The adenocarcinoma of the uterus was found at 700 days of age, when only five of the 30 rats had survived.

Elson¹¹⁴ dissolved 12 mg. of 2'-chloro-4-dimethylaminostilbene in 0.5 ml. of arachis oil and injected it into the peritoneal cavity of

ten female rats; five doses were given at about two-week intervals for a total of 160 mg. After 589 days one rat was found to have a carcinoma in the uterus.

Charkviani,¹¹⁵ using castrated albino rats, produced papillomatous foci in the uterus with Synestrol[®] alone. He also found a few carcinomas of the uterine horns.

A number of other authors¹¹⁶⁻¹²⁵ applied estrogenic stimuli to rats and obtained glandular hyperplasia, squamous metaplasia, and often hydrometra and pyometra.

Mice.--Loeb et al.¹²⁶ treated an "Old Buffalo" female mouse with estriol, starting when the animal was 18 days old. For the first 18 months 10 R.U. of estriol in water were given, and for the following six months the dose was increased to 30 R.U. Adenocanthomas were seen in the uterine cervix and horn.

Lacassagne¹²⁷ gave alternate administrations of estrone and hypophyseal extract each week to seven mice of two litters of Line 37, which had its origin in CBA strain mice. Treatment was started at the age of two weeks, and after 164 days one mouse developed a squamous cell carcinoma of the uterus and died. This tumor arose from the distal segment.

A number of experiments in carcinogenesis with estrogenic substances on mice have produced particularly mammary carcinomas,¹²⁸ squamous cell carcinomas of the uterine cervix,^{129,130} and leukemia. Some of these malignant diseases were probably spontaneous tumors of viral origin promoted by hormonal action. Endometrial tumors were definitely in the minority.

A few other investigations, in which the administration of estrogen to mice was relatively prolonged and no carcinomas of the endometrium were produced, are listed in our references.¹³¹⁻¹³⁴

Gardner and Ferringno¹³⁵ noted subserous uterine tumors, often multiple, in estrogen-treated mice, particularly in multiparous mice. They considered these tumors to be endometrial adenomas.

Barbieri, Olivi, and Sacco¹³⁶ treated 72 female mice of BALB/CfC3H cb/Se strain with estradiol benzoate; 50 were non-castrated virgins and 22 were castrated at one month of age. An oil suspension containing 0.05 mg. of estradiol benzoate was injected subcutaneously each week for seven to 24 weeks. The non-castrated group received the first treatment when 35-40 days of age; the castrated group were two months old. Of the 72 mice, 54 developed mammary carcinoma. The remaining mice were killed at 250 days; seven showed microscopic carcinomas of the breast and six had carcinomas of the uterus. All the uterine carcinomas appeared in the non-castrated virgin females; four were adenocarcinomas, three of which were in the body of the uterus and one in the cornua and cervix. One tumor was a mixture of adenocarcinoma of the corpus and squamous cell carcinoma of the cervix. One tumor was solely squamous cell carcinoma of the cervix.

Van Nie et al.¹³⁷ implanted 1-2 mg. of testosterone propionate into the subcutaneous tissue of BDF₁ female mice twice weekly. The average survival time was 15-18 months. Of the 42 mice so treated, 26 showed uterine sarcomas. Most of the tumors were seen at the cervix.

The Effect of Chemical Carcinogens upon the Endometrium

Rabbits.--Orr and Polson²⁸ observed an adenocarcinoma of the endometrium in three rabbits that had received chemical carcinogens. In view of the sites of application of the carcinogenic substances and the duration of the experiments, it is more likely that these tumors were spontaneous rather than experimental in nature, as the authors suggested.

Burrows and Boyland⁵⁴ regarded all endometrial carcinomas observed in their experiments with chemical carcinogens as spontaneous.

Troitskaya-Andreeva¹³⁸ applied 2-15 mg. of 3-methylcholanthrene in paraffin oil into the uterine horns of 50 rabbits. In an animal receiving 5 mg. a proliferating adenoma which reached to the serosal surface was found on the 1005th day. Two uterine sarcomas were also found.

Schinz and Steinman¹³⁹ performed laparotomy on three pregnant rabbits and injected 0.25-0.5 ml. of a 1 per cent solution of 3-methylcholanthrene in sesame oil into the fetus. The animal surviving for four years had an adenoma of the uterus.

Müller¹⁴⁰ reported three adenocarcinomas found in three rabbits weighing 2.5 kg. into whose ovaries benzpyrene had been implanted. These tumors were probably spontaneous in nature.

To eight rabbits Bonser and Green¹⁴¹ gave orally 100 mg. of powdered 2-acetylaminofluorene twice weekly until the animals died. After four years of treatment with a total dose of 42 g., one rabbit developed an adenocarcinoma of the endometrium.

Merriam et al.⁵⁷ inserted strings to which 3-methylcholanthrene

in beeswax (1:3 suspension) had been applied into the right uterine horn of 15 adult rabbits weighing 8-13 lbs. The rabbits were followed by a series of biopsies until sacrificed. After 5-7 months 11 of 12 rabbits showed adenomatous hyperplasia, and between six and 11 months seven of the eight survivors developed precancerous lesions. At the conclusion of the experiment (10-11 months from time of initiation) seven uterine tumors were obtained; of these, five were adenocarcinomas, one an adenoacanthoma, and one a carcinosarcoma. The left horns of half these animals showed cystic and/or adenomatous hyperplasia. Since at the start of this experiment two spontaneous carcinomas were found, the results of this experiment are difficult to assess.

Griffiths and co-workers¹⁴² introduced loosely woven cotton strings impregnated with 3-methylcholanthrene dissolved in beeswax in a ratio of 1:3 into the uterine horns of New Zealand white female rabbits 9-12 months of age. In one group of animals, weekly 1.25 mg. injections of Delalutin[®] (hydroxyprogesterone caproate) were started at the time the strings were inserted; in another group the injections were started seven months after string insertion; in a third group, ten months. Of the 29 rabbits receiving such combinations of treatment, eight developed carcinoma in the year this experiment was continued. In a group of eight castrates that received the methylcholanthrene-impregnated strings but no Delalutin one carcinoma developed. A group of 18 normal rabbits developed ten carcinomas when methylcholanthrene alone was applied.

Rats.--Castellano and d'Amour¹⁴³ administered 3-methylcholanthrene, and this carcinogen plus various hormones, to University of Denver

strain white rats whose spontaneous tumor incidence was known to be practically zero. At three months of age the animals were given the chemical in 20 per cent suspension into the uterine cavity, the total dose per animal being about 10 mg. Various hormones were given three times weekly. In the 89 rats thus treated 22 uterine tumors developed. Of the 20 rats receiving methylcholanthrene alone, two developed tumors. The average time of tumor appearance, detected by palpation, was 180 days. In the group of ten castrated females, one tumor developed, at 257 days. In nine rats receiving stilbestrol, 5 μ g. three times weekly, six tumors appeared at an average time of 179 days; of ten animals given 15 μ g. of the hormone, seven developed carcinomas of the uterus at an average 173 days; of the ten rats that received 5 μ g. of stilbestrol together with 5 mg. of progestin three times weekly, one had a tumor at 226 days. With progestin, 5 mg. three times weekly, one of ten rats showed a tumor at 192 days. One of the ten rats given 1 I.U. of P.U. gonadotropin had a tumor at 251 days; with 1 I.U. of P.M.S. gonadotropin, three of ten animals had uterine tumors at an average 140 days. 3-Methylcholanthrene was also applied on the ovaries of 20 other rats, ten of which also received P.M.S. gonadotropin 1 I.U. three times weekly, and one developed a tumor at 51 days. No histologic details of the tumors were mentioned.

Charkviani¹⁴⁴ produced endometrial carcinomas in non-castrated virgin female rats 4-5 weeks old at start of the study by the introduction into each uterine horn of a paraffin stick containing 1 mg. of 9,10-dimethyl-1,2-dibenzanthracene. The experiment lasted 502 days. Precancerous changes appeared between the 90th and 120th days, and the

first malignant tumor was obtained on the 120th day. Of 63 rats, 56 survived this "tumor age" of 120 days and 55 of them developed malignant uterine tumors. In histologic type, 15 were adenocarcinomas, 27 squamous carcinomas, seven undifferentiated carcinomas, and six were mixed type.

In a second series of experiments Charkviani¹⁴⁵ used castrated rats of the same age as in the previous study, and in addition to the paraffin sticks containing the carcinogen, some animals were given 0.1 mg. of Synestrol^(R) every fifth day. Of 63 animals receiving the carcinogen alone, 58 survived the tumor age of 120 days and 42 developed tumors; eight were adenocarcinomas, 14 squamous carcinomas, 11 mixed type, and four were undifferentiated carcinomas; the other six were sarcomas. Of the 54 rats that in addition received the estrogen, 44 survived the tumor age and 16 tumors were obtained (five adenocarcinomas, eight squamous carcinomas, two mixed, and one undifferentiated). In the first group the tumors developed after 5-5½ months, in the second group after 6-7 months. Soon after introduction of the carcinogen, marked inflammation was noted. Atypical epithelial change occurred between 160 and 200 days.

In a third series of experiments, lasting 440 days, Charkviani¹⁴⁶ castrated the rats and inserted the carcinogen as before. One group of animals then received in addition 5 mg. of testosterone propionate every fifth day, while a second group were given hypophyseal irradiation in total doses of 2,500-5,000 r. In the first group, 55 rats survived beyond the tumor age and 31 developed tumors. The first tumor was discovered on the 156th day. In the group receiving radiation,

30 of 54 survivors had tumors. These figures include tumors developing in the cervixes as well as those arising in the horns. Of the 61 tumors produced, 20 were adenocarcinomas, 37 were squamous, and four were undifferentiated.

Kupperman and Greenblatt¹⁴⁷ reported negative results with the local application of 3,4-benzpyrene.

Because of their strong response to the oral carcinogens, numerous experiments have been performed on rats with those chemicals. Some of such experiments induced a few endometrial carcinomas. Since this dissertation is mainly concerned with the local application of the chemical carcinogen, only brief descriptions of the previous results will follow.

By feeding 2-acetaminofluorene Bielschowsky¹⁴⁸ observed one uterine carcinoma in a rat of the Wistar substrain, Hoch-Ligeti¹⁴⁹ two uterine carcinomas in albino rats, and Morris, Dunn and Dubnik¹⁵⁰ one carcinoma in a Buffalo rat. By implantation of the chemical Wilson¹⁵¹ induced two endometrial adenomas. Walpole, Williams and Roberts¹⁵² observed two uterine carcinomas following 4-aminodiphenyl injection. Miller et al.¹⁵³ noted one adenocarcinoma in a Holtzman rat after the oral administration of 7-fluoro-2-acetylaminofluorene. Morris, Velat and Wagner¹⁵⁴ produced three carcinomas with the feeding of N-2-biphenylacetamide in Buffalo rats. Morris et al.⁷² obtained nine adenocarcinomas and two carcinosarcomas of the uterus by the feeding of fluorene and its derivatives in the same strain of rats. Druckrey and Schmähl¹⁵⁵ produced one adenocarcinoma in a BDVI rat with the oral administration of anilin-azo-2-naphthylamine, another with

1-o-tolylazo-2-naphthylamine in a BD III rat. Morris et al.⁷³ induced three adenocarcinomas and one adenoacanthoma of the endometrium in Buffalo rats with the feeding of N,N'-fluorenylenebisacetamide. They also noted three adenocarcinomas in AxC rats given the same chemical. Miller et al.¹⁵⁶ noted an adenocarcinoma of the endometrium in a Holtzman rat following the oral administration of 2,4-bis(acetylamino) fluorene. Morris et al.^{157,158} obtained five carcinomas of the endometrium with N,N'-2,7-fluorenylenebisacetamide and another five with N,N'-2,7-fluorenylenebis-2,2,2-trifluoroacetamide in Buffalo rats.

Since the 1940's, the peculiar carcinogenic property of organic polymers has been demonstrated by many investigators, and a large number of references can be found in some recent publications.^{159,160}

Hueper¹⁶¹ noted a high incidence of uterine cancers in polyvinyl pyrrolidone-treated Bethesda black rats. Of 300 rats which received this chemical by subcutaneous, intraperitoneal, or intravenous route, 16 uterine carcinomas were observed, and 13 were adenocarcinomas and three squamous cell carcinomas, histologically. In his later experiments with the same strain of rats,^{69,70,160} he observed frequent uterine carcinomas, which were either adenocarcinomas or squamous cell carcinomas. Occasionally a mixture of the two types of carcinomas and carcinosarcomas were seen. The substances that were applied to rats and produced tumors include: polyvinyl pyrrolidone, polyvinyl alcohol, dextran, silastic rubber, silicone, and polyurethane.

Several experiments that produced uterine sarcomas in rats are listed in our references.¹⁶²⁻¹⁷⁰

Mice.--Ilfield,¹⁷¹ by injecting 5 per cent 1,2,5,6-dibenzanthracene

in cholesterol into the uterus of 21 mice, produced one squamous cell carcinoma.

Perry¹⁷² applied 0.3 per cent 1,2,5,6-dibenzanthracene in benzene and 0.05-0.1 per cent estrone in benzene to the skin of normal and castrated mice. Over a period of ten months three squamous carcinomas of the cervix were obtained out of 27 mice that had survived beyond six months of treatment.

Kleinberg¹⁷³ produced a cystadenoma of the uterus in one of 21 R-U mice to which he had given three subcutaneous applications of 0.5 per cent 1,12-trimethylene crysene in 0.4 ml. of olive oil.

Van der Schueren¹⁷⁴ administered weekly 0.1 ml. of 3-methylcholanthrene in olive oil into the vaginae of 47 mice. One animal developed a uterine carcinosarcoma.

Pan and Gardner^{175,176} made subcutaneous transplants of virgin uteri from 98 inbred and hybrid mice, which contained small crystals of 3-methylcholanthrene, to homologous hosts. Thirty-one homografts developed carcinomas, 28 of which were squamous carcinomas and three adenocarcinomas. Some of the squamous carcinomas and all of the adenocarcinomas were derived from the uterine horns. The earliest tumor appeared two months after the transplant.

Bonser and Robson¹⁷⁷ applied 3-methylcholanthrene directly into the uterine cavities of CBA and white mice. Crystals of the carcinogen were injected into the uterine cavity. A suture was placed proximal and distal to the crystal and by this means 0.1 mg. of crystal was held within the uterine cavity. Some animals received 1 per cent 3-methylcholanthrene in lard injected through the vagina into the uterine

cavity. A slight modification was made in the first method whereby the tip of the horn was cut cranial to the proximal suture in order to reduce the incidence of sarcoma. The results of their studies are tabulated in Table 1.

Hall, Balder and Hamilton¹⁷⁸ ovariectomized over 200 young mature and mature CF 1 mice and implanted by trocar about 2 mg. of 3-methylcholanthrene into each uterine horn. Diethylstilbestrol, 20 μ g. in 0.05 ml. of oil, was given three times weekly up to 20 weeks. All animals died or had been killed by 180 days after implantation of the carcinogen. Nearly half the 57 mice that survived the estrogen treatment and lived at least 100 days developed uterine tumors, most of them in both cornua. The incidence of tumors was higher in the mice receiving the estrogen for longer periods and the tumors were especially large and aggressive in these animals. The ratio of sarcomas to carcinomas was estimated at 1:4.

Laffargue et al.¹⁷⁹ inserted beads of 3-methylcholanthrene into the uterine cervixes of 84 mice. Among 69 mice examined, 9 tumors were found originating from the uterine horns; one was an adenocarcinoma and eight were squamous carcinomas.

Kaslaris and Jull¹⁸⁰ injected pure carcinogens into the uterine horns of WLL and Strong A strains of mice four to six weeks old. With 3-methylcholanthrene, four adenocarcinomas and two squamous carcinomas were produced in 30 animals representing both strains. The three other chemical carcinogens used produced carcinomas only in mice of the Strong A strain. With 9,10-dimethyl-1,2-benzanthracene one adeno- and one squamous carcinoma were produced in 26 mice. 3,4-Benzpyrene induced

Table 1 - Experiments by Bonser and Robson (1950)

Type of mouse	Ovarian status	Carcinogen	No. of animals	Adeno-carcinomas		Adeno-acanthomas		Carcino-sarcomas		No. of tumor bearers
				No.	Mos. for develop.	No.	Mos. for develop.	No.	Mos. for develop.	
CBA immature	spayed	crystal	4	2	9	0		0		4
		in lard	2	1	8	0		0		2
	intact	crystal	9	1	15½	1	16	0		6
White mature	spayed	crystal	12	1	13	2	11,12	0		4
	intact	crystal	24	4	11,15,17,20	0		0		7
		in lard	9	1	13	0		0		2
White immature	spayed	crystal	14	1	17	0		0		1
	intact	crystal	13	2	5,8			1	5	4

One adenocanthoma had metastasized to a regional lymph node; otherwise the presence of metastasis was not mentioned.

only five tumors, all adenocarcinomas in 16 mice. Only Strong A strain mice were given injections of 1,2,5,6-dibenzanthracene, with which five adenocarcinomas were obtained from the seven mice used. In a second series of experiments these investigators administered 3-methylcholanthrene to CBA mice, some mature, others immature. From ten spayed immature mice receiving the carcinogen alone, three adenocarcinomas, two adenoacanthomas, and one squamous carcinoma were recovered. Fifteen spayed immature mice in addition to the carcinogen received 50 µg. of estradiol dipropionate every second week, and from these animals four adenocarcinomas, four adenoacanthomas, and three squamous carcinomas were obtained. When castrated mature mice were used, five adenoacanthomas developed in the 30 animals. No carcinoma developed in spayed mature animals treated with progesterone or testosterone.

Taki and Iijima¹⁸¹ inserted 3-methylcholanthrene-impregnated cotton strings into the uterine horns of na2 female mice about 12 weeks old. From 106 mice examined histologically, 58 atypias, 30 adenocarcinomas, five adenoacanthomas, two squamous cell carcinomas, and two sarcomas were obtained. All carcinomas were observed after eight weeks of methylcholanthrene application.

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CHAPTER II

MATERIAL AND METHODS

Rabbits.--Because of a high incidence of spontaneous endometrial adenoma and adenocarcinoma, the Dutch female rabbits, 4-5 years old, weighing 4-5 lbs., were used in these studies.

A comparative study of the development of endometrial carcinoma in animals receiving carcinogen alone, hormones alone, a combination of carcinogen and hormone, and control treatment was designed. The animals were divided into the following groups:

<u>Group</u>	<u>Treatment</u>	<u>No. of rabbits</u>
1	3-methylcholanthrene tampons	21
2	3-methylcholanthrene tampons and diethylstilbestrol injections	24
3	Diethylstilbestrol injections	78
4	Controls:	94
	a) Ethyl oleate injections	14
	b) Polyvinyl chloride and paraffin tampons	10
	c) No treatment	67
	d) Castrates	3

The tampons were prepared by dipping strips of cotton umbilical tape into a 20% suspension of 3-methylcholanthrene in solution of equal parts of polyvinyl chloride and melted paraffin. The approximate weight of the carcinogen associated with the string was 36 mg. (34-44 mg.). The operative procedure consisted of laparotomy and resection

of the tip of a uterine horn with insertion of the carcinogenic substance. Castration was not performed.

Injections of diethylstilbestrol were given weekly. Injections of 0.2-0.3 ml. of a 2.5% diethylstilbestrol in ethyl oleate were given into the back muscle of the rabbit. Data indicating the duration of injections are presented in Table 5.

All animals were examined each week for the presence of tumors in the abdomen. Animals appearing very sick or with abdominal masses were killed shortly after discovery of their condition. With the animal under ether anesthesia, a piece of endometrium, usually from the mesometrial side, was resected with a pair of scissors. In the 3-methylcholanthrene treated animals specimens were taken from both uterine horns for electron microscopy. The uterus, upper vagina, oviducts, and ovaries were resected in toto and fixed in 10% neutral buffered formalin. The animal was then killed by intracardiac injection of pentobarbital.

Fifty additional Dutch rabbits were kept for observation and received no treatment except for occasional administration of Sulmet[®] for upper respiratory infections.

Rats.--Bethesda Black female rats were obtained from the National Institutes of Health, Bethesda, Md. The mature animal weighed approximately 250 g. The animals were divided into four groups:

<u>Group</u>	<u>Treatment</u>	<u>No. of rats</u>
1	3-methylcholanthrene tampons	18
2	3-methylcholanthrene tampons and diethylstilbestrol injections	19
3	Diethylstilbestrol injections	19

4	Controls:	21
	a) Ethyl oleate injections	7
	b) Polyvinyl chloride and paraffin tampons	7
	c) No treatment	2
	d) Castrates	5

The injections of diethylstilbestrol were given in weekly doses of 1.25 mg. Data indicating the duration of injections are presented in Table 6. Approximately 0.05 ml. of a 2.5% solution of diethylstilbestrol in ethyl oleate was injected into the lumbosacral muscles. The tampons were prepared by dipping a cotton string in a 20% solution of 3-methylcholanthrene dissolved in a 1:1 mixture of polyvinyl chloride resin and melted paraffin. The dose of the carcinogen ranged from 2-6 mg. with an average of 3 mg. The operative procedure was similar to that described in the experiments on mice (to follow).

Another group of 98 rats of the JR strain were divided into the following groups:

<u>Group</u>	<u>Treatment</u>	<u>No. of rats</u>
1	3-methylcholanthrene tampons	22
2	3-methylcholanthrene tampons and diethylstilbestrol injections	22
3	Diethylstilbestrol injections	21
4	Controls:	33
	a) Ethyl oleate injections	10
	b) Polyvinyl chloride and paraffin tampons	9
	c) No treatment	14

These animals were treated in a manner similar to that just described.

The rats of the first colony were observed for the development of tumors. A few biopsy specimens were taken for study under the electron microscope, but most of the material was obtained at autopsy and used only for gross and light microscopy. Since almost all animals receiving the carcinogen, whether alone or in combination with hormone injections, developed tumors, they were all sacrificed. Six months after the experiments were started on the second colony, biopsy specimens were taken, fixed, and embedded in Maraglas for electron microscopy. Autopsies were performed on all dead animals. Animals with an abdominal mass (pyometra) or that became sick were sacrificed shortly after the discovery. When animals were found dead and much autolysis had occurred, only the female genital tract was saved and some were examined only grossly. When animals were sacrificed, the ovaries and oviducts were removed with the uterus and upper vagina, as were the thyroid, adrenals, and hypophysis, as with the rabbits.

Six rats of the second group of animals were castrated and biopsies were taken from these one month later for electron microscopy. Diethylstilbestrol injection was started on the survivors and continued on a weekly schedule for one month. The dosage was 1.25 mg. per week.

A third group of Bethesda Black rats consisting of 30 females was castrated and a string with carcinogens was inserted into the uterine cavity of each animal. Ten rats received 3-methylcholanthrene dissolved in an equal mixture (1:1) of paraffin and polyvinyl chloride resin. The second ten animals received 3-methylcholanthrene dissolved only in pure paraffin. The last ten rats received 9,10-dimethyl-1,2-benzanthracene dissolved in pure paraffin. The carcinogen was

dissolved at a concentration of 20%. One-half of the animals in each group received in addition to the operative procedure described, weekly injections of diethylstilbestrol in ethyl oleate. A single dose was 1.25 mg. The main purpose of this phase of the experiments was to obtain material for electron microscopy in order to analyze the early fine-structural changes occurring in the endometrial cells after the application of the carcinogen.

Mice.--B₆D₂F₁ virgin female mice weighing 12-16 g. (approximately 5-6 weeks old) were obtained from the Jackson Memorial Laboratory, Bar Harbor, Maine. This breed has black fur and is derived as F₁ hybrid between C57BL/6 female and DBA/2 male mice. It is of interest that the parent strain of C56BL has a very low incidence of mammary carcinoma. Thus B₆D₂F₁ mice were considered suitable for our experiments with estrogens.

The mice were kept five or fewer to a cage made of disposable plastic. Bedding material consisted of dried, crushed corn cobs. Food and water were given ad libitum. The food was Purina Laboratory Chow.

3-Methylcholanthrene in oil was injected into the uterine horn of 50 of these mice and the animals were castrated. The operative technic was clean but not strictly sterile.

For the first group of injections, 3-methylcholanthrene crystals were suspended in olive oil in concentration of 20 w/v per cent. The suspension was aspirated into a 1 ml. tuberculin syringe. The material was injected into the lumen of the uterus. A small amount of oil could be injected. The injection was done very slowly and gently, but in

spite of this there was often leakage of oil or rupture of the uterine wall. Nine of the 50 mice died in the early postoperative period, leaving 41 animals to be kept under observation.

Each month for seven months four animals were killed and tissues were fixed for electron microscopy. All tissues were examined by light microscopy. Animals found dead were kept in 10% formalin and the uteri were examined after the tumors and sections were taken for the morphological study.

In a second series strings impregnated with the carcinogen were inserted in the uterine horns of 64. Seven died in the postoperative period and 57 were kept under observation. Pure 3-methylcholanthrene crystals were melted and painted on cotton string, and the insertion of pieces of this string 4-5 mm. in length was substituted for the injection of the oil suspension used in the former series. The uterine horns were tied both proximal and distal to the string.

Since in the second series of the experiment many of the strings painted with the carcinogen slipped into the peritoneal cavity, a third series of 47 animals was started to find a more satisfactory method of introducing the 3-methylcholanthrene. To avoid the slipping of the strings, sutures were no longer placed in the distal segment of the uterine horn. The 3-methylcholanthrene was applied in several media on 5-0 black surgical silk, both ends of which were tied to the uterine wall in both horns so that the string passed through the lowest uterine segment in the midline. As media, the following materials were used: paraffin alone, and a 1:1 mixture of paraffin and polyvinyl chloride, each of these media containing 20% of 3-methylcholanthrene.

The latter mixture was also absorbed on small pieces of Gelfoam (Upjohn Co.), which were inserted into the uterus. Pure 3-methylcholanthrene was also applied directly on strings. When strings coated with the carcinogen in paraffin or in the mixture of paraffin and polyvinyl chloride were inserted by the method described above, the difficulty encountered in the second series of the experiment was overcome. Only a few of the animals used in this experiment were killed; the rest were kept under observation for the development of tumors.

Thirty-six mice were started on weekly injections of 0.025 ml. (5 mg.) of diethylstilbestrol in 1 ml. of vegetable oil (Eli Lilly Co.). With a 25 G. needle the solution was injected into the subcutaneous tissue of the back of the mice. Animals were killed every month after three months of administration, and the tissues were fixed and embedded in Maraglas for electron microscopy.

A fifth group consisting of 99 mice were not castrated, and were divided into groups to receive treatment as follows:

<u>Group</u>	<u>Treatment</u>	<u>No. of mice</u>
1	Thread of black surgical silk coated with carcinogen in 1:1 polyvinyl chloride resin and paraffin mixture	13
2	Thread with 3-methylcholanthrene as above plus weekly injections of 0.125 mg. of diethylstilbestrol	40
3	Weekly injections of 0.125 mg. of diethylstilbestrol in 0.025 ml. of vegetable oil	11
4	Controls:	30
	a) Thread control (paraffin and polyvinyl chloride resin without carcinogen	20

b) Injection control (vegetable oil
without diethylstilbestrol) 15

The free end of the black surgical silk was tied around the cut end of the uterus to secure it. The dose of the carcinogen was about 0.3-1 mg. per string. At the beginning and at the end of the experiment a few animals were killed and tissue was fixed for electron microscopy. Most of the animals were kept under observation for the development of tumors, determined by biweekly palpation of their abdomens, and autopsies were performed on all dead animals. For histological examination, the uteri without tumors were fixed and embedded in toto.

Biopsies were taken from the endometrium of five castrated mice, and then the administration of diethylstilbestrol was started with weekly injections of 0.125 mg. per dose. They were sacrificed two months later and tissue was obtained for electron microscopy.

In the sixth series, 68 female B₆D₂F₁ hybrid mice with the specified body weight of 12-15 g. were castrated, and at the same time strings with carcinogens were inserted into one of the uterine horns. The first group of ten animals received strings with 3-methylcholanthrene dissolved in an equal mixture of paraffin and polyvinyl resin at a concentration of 20%. The second group of animals were inserted with strings of 3-methylcholanthrene which had been dissolved in pure paraffin alone. The last group were treated with strings prepared with 9,10-dimethyl-1,2-dibenzanthracene mixed in paraffin. Half of the animals in each group were treated further with weekly injections of diethylstilbestrol in oil solution. A single dose of 0.125 mg. was injected subcutaneously each week. This part of the experiments was

designed primarily to obtain material for electron microscopy, and one animal from each of the six groups, each group representing a different type of treatment, was sacrificed every two weeks. The remaining eight animals underwent castration alone without further treatment.

The total numbers of animals are summarized in Table 2.

Preparation of Material for Light Microscopy

The tissues obtained from the uterus and other organs were fixed in 10% neutral buffered formalin, embedded in a paraffin-wax mixture, and sections ranging from 4-6 μ in thickness were cut. The uterine tissue from the mice was trimmed to a level where the cervix or the lumen could be adequately shown. All sections were stained with hematoxylin-eosin stain.

Preparation of Material for Electron Microscopy

While the uterine circulation was maintained, tissue measuring approximately 1 x 1 x 1 mm. was removed and placed on a glass plate with the luminal aspect of the tissue up. The surface was immediately covered with fixative and the tissue dissected into ten or more small pieces. These pieces were then transferred into a vial containing approximately 1.5 ml. of ice-cold fixative and stored in a refrigerator at 4-6° C. The fixation time for the mouse and rat endometria was 60 minutes, for the rabbit tissue, 90 minutes. Two fixatives were used in our experiments; in the later experiments the second fixative was used for all tissues.

1. Palade solution⁷ (1% osmium tetroxide in barbital buffer). The solution was prepared according to Caulfield's method⁸ just before use.

Table 2. Material

ANIMAL	RABBIT (Dutch)	RAT (Bethesda Black)	MOUSE (B6D2F ₁)
Normal control	117	16	24
Castrate	3	5	13
Diethylstilbestrol	78	40	47
Carcinogens	21	56	204
Carcinogens + Diethylstilbestrol	24	56	70
String control	10	16	20
Injection control	14	17	15
Total	267	205	393
		865	

2. Millonig solution⁹ (1% osmium tetroxide in phosphate buffer). This fixative appeared to penetrate the tissue better than the Palade solution, and the staining property of both thick sections for light microscopy and of thin sections for electron microscopy was markedly enhanced.¹⁰

In the early experiments the tissues were embedded in either methacrylate resin¹¹ or Epon 812 resin.^{12,13} In later experiments Maraglas mixture^{14,15} was used for most tissues.

Sections 1 μ thick were cut and stained for light microscopic identification of the area to be later sectioned for electron microscopy. The sections were cut with a diamond knife on a Porter-Blum ultramicrotome.

Blocks containing either surface or glandular epithelium or both, preferably tangentially cut areas, were chosen for thin sectioning. Sections approximately 1/20-1/10 μ thick were picked up on uncoated 300-mesh copper grids.

For light microscopy the 1 μ sections were stained with toluidine blue.^{16,17} For electron microscopy the sections were stained with a saturated solution of uranyl acetate in 70% ethyl alcohol for 20-30 minutes, followed by lead citrate for times that varied with the fixative. Tissue fixed in Millonig's fixative⁹ were stained for 5-10 minutes; tissue fixed in Palade's fixative were stained for 30-60 minutes. Other lead staining techniques¹⁸⁻²⁰ were also tried but were not as reliable or satisfactory as the combination of uranyl acetate-lead citrate method.

The sections were examined in an Akashi Tronscope TRS-50 equipped

with a 100 μ objective aperture and in an RCA-EMU-3F electron microscope equipped with a 50 μ objective aperture and a double condenser lens.

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CHAPTER III

RESULTS

Rabbit

Normal endometrium of the rabbit.--Since most of our rabbits were old animals, as attested by the fact that many of them suffered from moderate to severe arteriosclerosis of the aorta, their endometria showed a rather interesting variety of patterns.

A normal endometrium is one that must satisfy the following criteria: (1) good differentiation of surface and glandular epithelium, (2) regularly distributed glands without hypersecretion, (3) no cellular and/or architectural abnormalities, (4) a moderate amount of well-developed stroma, and (5) the absence of significant inflammation. Papillary or polypoid changes of the superficial epithelium should not be observed.

As seen with the light microscope (Fig. 1), the endometrial surface was flat, and both ciliated and non-ciliated cells were distributed, usually in clumps, among the surface epithelium. In some areas the ciliated cells predominated, in others only non-ciliated cells were seen. In the ciliated cells the luminal cell borders were usually eosinophilic. The amount of cytoplasm in a surface cell varied from cell to cell. Often cells showed slight basophilia. The nucleus was usually pale and was located in the basal portion of the cell or sometimes in the middle portion.

In the deep portion there were usually many evenly distributed

glands surrounded as a rule by smaller cells with fewer cilia and less secretory activity than those of surface cells. In general, the glandular cells had paler cytoplasm and the nuclei were basally located.

With the electron microscope, ciliated (Fig. 3) and non-ciliated cells (Figs. 2 and 4) could be differentiated. The non-ciliated cells in both surface and glandular epithelium showed minimal secretory activity. In the apical cytoplasm there were a few membrane-bound vesicles with scanty granular material or dark homogeneous material in the center. The vesicles in the vicinity of the luminal cell surface were distended, the contents was thin, and the membrane was incomplete. The luminal surface of the non-ciliated cells was lined by many microvilli, while the ciliated cells had both cilia and some microvilli. The rootlets of the cilia were not cross-striated. The lateral cell membranes were straight with occasional tortuosity. In the apical portions there were many desmosomes and terminal bars, and the intercellular space was very regular. The apical cytoplasm contained most of the organelles. Mitochondria showed fairly regular cristae. The mitochondrial matrix was faintly electron-dense and the intramitochondrial microbodies were small. The Golgi apparatus was well-developed and often contained slightly electron-dense material; the cisternae were swollen. The ergastoplasm was often dilated and contained faintly stained material. In many places the ergastoplasm was incompletely granulated and many clumps of free ribosomes were seen. In the glandular cells there were many membrane-bound dense bodies 170-700 μ in diameter. Many microvesicular bodies were seen in the apical cytoplasm of the glandular cells. Cytoplasmic fibrils were

very inconspicuous. Small vesicles containing electron-dense homogeneous material and measuring 20-70 mu in diameter were distributed throughout the cytoplasm. In the middle and basal portions of the cytoplasm the cells contained mitochondria, free ribosomes, and rough-surfaced ergastoplasm. Secretory granules, Golgi apparatus, and lysosomes were less frequent. The nuclei were relatively large, occupying half the cell height. The space between the outer and inner nuclear membranes was only slightly widened. There were no excessive invaginations of the nuclear inner membrane. The karyoplasm was of moderate density and rather homogeneous. Condensation of chromatin was seen around the nuclear membrane, more in the glandular cells. Nucleoli were moderate in size, and most cells showed one or two nucleoli.

The ultrastructure of the cells of the upper glandular epithelium was almost identical to that of the superficial epithelium. However, in the deeper portions of the epithelium ciliated cells were much fewer. The majority of the cells were similar in appearance and were non-ciliated (Fig. 5). There was minimal secretory activity. The luminal cell surface was very short and was covered with a number of microvilli having a more complex appearance than those in the surface cells. Occasionally a fusion of microvilli was suggested. The lateral cell membranes were relatively straight, showing far less tortuosity than those of the surface cells. The intercellular spaces were very narrow.

Most of the cytoplasmic organelles were present in the apical portion of the cytoplasm and were similar to those present in the surface cells. Free ribosomes were numerous. Many multivesicular bodies were seen in the subluminal layer. A few secretory vesicles containing

fibrillar or dense material were seen which measured 300-400 μ in diameter.

The nuclei of the glandular cells were darker than those in the surface cells since the karyoplasm was less evenly distributed and moderate chromatin condensation was found, especially next to the inner nuclear membrane. Smaller and less dense condensation was also noted in the inner portion of the nucleus. No difference could be recognized in the morphology of the nucleolus from that in the surface epithelial nucleus.

Castration effect.--The uterus of the castrated rabbit showed remarkable atrophy which consisted mainly in a reduction of the diameter of the uterine horns, largely due to shrinkage of the myometrium. The uterine horns averaged no more than 5 mm. in diameter. The endometrial cavity was very small and the mucosal surface was rather smooth. The endometrium was thin.

The histological preparations showed a marked reduction in size of the cells in the superficial epithelium (Fig. 6). This was mainly due to the loss of the apical cytoplasm. Most of the surface cells appeared cuboidal. Ciliated epithelial cells predominated while the population of non-ciliated cells seemed reduced. The glandular spaces were fewer and smaller. The glandular spaces deeper in the endometrium had disappeared. Although rare glands showed some cystic dilatations, the majority of the glands possessed very small lumens.

In the electron microscope, the surface cells might be ciliated or non-ciliated whereas none of the gland cells were ciliated.

The ciliated cells were relatively increased (Fig. 7). The

cytoplasm between a nucleus and lumen contained a few slender mitochondria. Elements of the ergastoplasm, rough- and smooth-surfaced variety, were present in small numbers. The Golgi apparatus was of a moderate size. Several lysosomes up to $2\ \mu$ in diameter were generally present. The larger lysosomes might contain lipid material, the smaller ones many vesicles. Also present in this area were clumps of glycogen granules and many free ribosomes.

The basal part of the cytoplasm contained mitochondria, which were occasionally moderately crowded beneath the nucleus, and areas of glycogen accumulations. Some of the mitochondria were more than $4\ \mu$ in length. The other organelles included vesicles of the rough-surfaced ergastoplasm, a few lysosomes, and many ribosomes. Lipid droplets were not present.

The nucleus was elongated, usually elliptical, and the nucleocytoplasmic ratio was high.

Non-ciliated cells were present in groups on the endometrial surface. The cells in these groups were shorter than the ciliated cells. The luminal cell surface contained a few small microvilli, shorter than the microvilli of the ciliated cells. The apical cytoplasm contained few mitochondria and a poorly developed Golgi apparatus. Mitochondria were not as slender as those of the ciliated cells. There was more rough-surfaced ergastoplasm than seen in the ciliated cells. A few thick-walled vesicles were present ($0.35\ \mu$ or less in diameter). Lysosomes and multivesicular bodies were also present. Some cytoplasmic fibrils were seen. The basal cytoplasm contained slightly larger and wider mitochondria and less rough-surfaced ergastoplasm,

which was occasionally swollen. Vesicles of less than 40 μ were numerous. Glycogen granules were not observed. The nucleus was large with high nucleocytoplasmic ratio. Nucleoli were prominent and the nucleoplasm was dense.

Cells deep in the glands were shorter than the cells in the upper gland, and the nucleocytoplasmic ratio was high (Fig. 8). Their luminal surface contained few microvilli. The basal lateral cell border was not tortuous but parallel to the apposing cytoplasmic membrane. The intercellular space was often widened.

The apical cytoplasm contained large round mitochondria. Many vesicles with thick walls and measuring 200 μ were seen. The Golgi apparatus and many ribosomes were present. Elements of the rough-surfaced ergastoplasm were scarce. Some of the smooth-surfaced reticulum was swollen and had the appearance of small vacuoles with sharp membranes. A faintly opaque material was present within these vacuoles. In the basal cytoplasm there were large swollen mitochondria. Many large lysosomes measuring 6-14 μ were seen. They contained lipid, membranous structures, and vacuoles. Apparently these lysosomes were in close contact with mitochondria.

The nucleus was large with distinct indentation. The nucleoplasm was thicker beneath the membrane, and the nucleoli were prominent.

Estrogenic effect.--The uteri of rabbits treated with estrogen were grossly hypertrophic with thickened wall, hyperemic with increased numbers of engorged capillaries, and edematous. The endometrial cavity was widened and the endometrial surface was coarse and occasional cystic change was noted. The peritoneal surface often showed slight fibrosis.

In the normal estrus the endometrial epithelium consists of ciliated and non-ciliated cells. The non-ciliated cells had secretory function (Fig. 9).

Under estrogenic stimuli, the cells were moderately elongated although occasional cuboidal cells were seen. The cells were tall but the epithelium was not unduly crowded with cells. The lumen was slightly widened but the dentate appearance was well preserved.

In the surface epithelium, the luminal cell surface was rather flat and sharp in the ciliated cells and stained slightly darker. Cilia could be identified. The cell membrane was distinctly darkly stained and thus differed from that of non-ciliated cells.

The non-ciliated cells had a smooth but rounded luminal cell surface. Secretion was recognized (Fig. 10). The cytoplasm in the ciliated cells was eosinophilic and stained rather darkly. No vesicles or granular inclusion material was noted. The supranuclear cytoplasm in the non-ciliated cells showed vesicles containing grayish mucoid granular material. Dense granular material was present around the nucleus. The nucleus of the non-ciliated cells was usually located at the base of the cell. Chromatin network was dense. The ciliated cells had a denser chromatin pattern and the nuclei were often found in the middle of the cells.

In the glandular epithelium there were both ciliated and non-ciliated cells. Generally the cells were taller than those on the surface and the cytoplasm appeared paler, particularly in the ciliated cells. The secretory activity of the non-ciliated cells seemed active.

In the estrogen-treated animals, there were always marked

proliferation and distention of capillary and venular beds, the stroma was moderately edematous. The arterioles had thickened walls, and a denser concentric arrangement of fibrocytes was noted.

In the electron microscope, the rabbit endometrium showed active mucus secretion but only in the non-ciliated epithelial cells. The surface cells were much elongated.

The apical cell surface of the non-ciliated cells protruded markedly into the lumen (Fig. 11). The protruding portion measured $7\ \mu$ in maximum and the microvilli were correspondingly markedly decreased in number and size. The basal portion of the lateral cell membranes was tortuous and the intercellular spaces were markedly widened, measuring 350-700 μ . In these widened intercellular spaces was a small amount of slightly electron-opaque material. The basal cytoplasmic membrane was wavy.

The apical cytoplasm of the mucus-secreting cells was almost filled with secretory products in the form of single-membrane-bound globules with an average diameter of $0.9\ \mu$. The contents of these globules seemed to be affected on fixation and consisted of an accumulation of irregularly interweaving fine fibrils with occasional round, swollen ends. Closer to the lumen, the membrane gradually dissolved and the lining of globules was incomplete, so that just below the luminal cell surface these secretion globules coalesced. There was no defect on the luminal cell surface but the surface of the secretory cells was much thinner and more irregular, compared with that of the ciliated cells. It was therefore presumed that the mucus diffuses out from the cytoplasm through the morphologically unbroken cell membrane

(an ecchrine pattern). In the vicinity of the nucleus there were many Golgi apparatus, mitochondria, multivesicular bodies (Fig. 12). In the apical portion these organelles were compressed in the center of the cells and the membrane-bound secretory globules occupied the periphery. As is usual in rabbits, the mitochondria were slender and measured 2.5μ in maximal length and $170 \text{ m}\mu$ in diameter. The cristae were regular. Some of the Golgi tubules, as well as some of the vesicles, contained a moderate amount of electron-dense material. Smooth-surfaced ergastoplasm was small and contained some homogeneous electron-opaque material. Rough-surfaced ergastoplasm was moderately well-developed and very narrow. No lysosomes and very few phagosomes were seen. The cytoplasmic fibrils were scattered and very fine. Some of them were connected to the desmosomes.

The ciliated cells were equally enlarged under estrogenic stimulation. However, because of the cilia and the attached basal bodies and rootlets, the luminal cell surface maintained its straight contour. Microvilli were elongated between the cilia. Numerous mitochondria were seen in the cytoplasm and showed well developed, regular crista formation. The Golgi apparatus was also well developed, but there was no swelling of its cisternae as in the mucus-secreting cells. The ergastoplasm was seen throughout the cytoplasm; its lumens were narrow probably due to compression by the swollen cytoplasmic ground substance; they had an average diameter of $40 \text{ m}\mu$. Generally, moderate ribosomal granulation was seen. Free ribosomes were in the usual clumps which were rather uniformly distributed throughout the cytoplasm. The distance between ribosomal clumps was, however, much

greater than in the normal inactive endometrium. This in all probability was due to the fact that the increment of the cytoplasmic ground substance was out of proportion to the increase of ribosomal clumps. After a long estrogen treatment, however, the ribosomes in some cases showed a rather dense distribution. No glycogen granules were seen.

In the deeper portions of the glandular epithelium only non-ciliated cells showed a minimal amount of secretion. The general features of these cells simulated the estrogen-stimulated non-ciliated cells since many cytoplasmic organelles were noted.

After a prolonged treatment with diethylstilbestrol, most cells retained quite good organization in their cytoplasmic structures. In some cases there was excessive cellular swelling in addition to the cellular enlargement. The cytoplasm became highly transparent, and the mitochondria and the lumens of the rough-surfaced ergastoplasm were markedly dilated. On the other hand, fewer secretory vesicles were seen.

Development of carcinoma.---Following the local application of cotton tapes impregnated with 3-methylcholanthrene, the uterus showed gross evidences of the effects of the carcinogens. The gross appearance of the endometrial surface, unless infected, was quite characteristic. Where no carcinogens had been applied (on the contralateral side) the surface had the usual pattern of tissue reaction to estrogens; the mucosa was hyperemic and edematous with small cyst formations and often was nodular and sometimes had small papillary projections. In the uterine horn into which the tapes impregnated with carcinogens had been inserted, the mucosa lacked such reactions. The endometrial tissue

was rather firm with little hyperemia or edema. The mucosal surface did not have a cystic appearance nor were papillary projections present. Instead it was very finely granular, simulating a cobblestone appearance. The thickness was definitely reduced.

Squamous metaplasia was seen in rare instances; it was very slight and was limited to the superficial epithelium (Fig. 13). The epithelium had more than three layers of cells that were very tightly adherent to each other, particularly in the luminal layer. The subluminal zone of the cytoplasm was very intensely eosinophilic. In general, the superficial epithelial cells became slightly enlarged, assumed a cuboidal shape, and showed increased eosinophilia of the cytoplasm. The nucleus was slightly enlarged.

The histological changes of the dysplastic stage were mainly in the surface epithelium and were much less apparent in the glands (Fig. 14). The surface epithelial cells were more enlarged and more intensely eosinophilic than the metaplastic cells. The cytoplasm often looked finely granular. The cells were cuboidal and were usually aligned in a single layer. Often a separation of the cells was noted. The nucleus was also enlarged and appeared vesicular. The nucleoli became very prominent. The underlying stroma showed moderate edema and hyperemia with numerous neutrophilic and eosinophilic leukocytes. The fibrocytes were enlarged and showed thick basophilic cell bodies. No appreciable change occurred in the myometrium. Most of the glandular epithelial cells showed no changes but in a rare gland were slightly increased in number. In these areas the cells were usually tall and

had a slight hyperchromasia of the nuclei, but there was no loss of cellular polarity.

In the electron microscope, there were marked cytoplasmic changes in the surface epithelial cells in the dysplastic stage. These cells had no cilia or secretion; they were often piled up in a pseudostratified pattern (Fig. 15). They were taller and broader. Some cells showed a reduction of the cytoplasmic organelles. And a decrease of the electron density of the cytoplasmic ground substance added to their appearance of emptiness. The luminal cell surface had a moderate number of microvilli. The lateral cell membrane was moderately tortuous, and a marked cellular dissociation with large intercellular spaces was noted. In the apical and middle portions of the cells only desmosomes connected the apposing cells. The desmosomes were very prominent, were enlarged and increased in number. They were also connected with the cytoplasmic fibrils. The more pronounced cellular dissociation in the basal portion of the epithelium was due to lack of desmosomes in this area. The basal cell membranes showed the formation of hemidesmosomes, which are one of the features of squamous epithelium.

The subluminal layer of the cytoplasm was often structureless and electron-dense with only moderately condensed cytoplasmic fibrils. Free ribosomes were decreased. The rough-surfaced ergastoplasm seemed also less developed. The lumens of the ergastoplasm were slightly dilated. The Golgi regions were smaller. There were many empty small vesicles in the apical cytoplasm that were, however, slightly larger than the Golgi vesicles and might represent vesicles of the smooth-surfaced ergastoplasm. The mitochondria in the superficial layer of

the epithelium were rather short and slender, although partial swelling of the mitochondria was commonly noted (Fig. 16). The defective construction of mitochondria was very frequently noted in the surface epithelial cells. The cristae were markedly decreased in number. The matrix was homogeneous, but the intramitochondrial bodies were large. The areas where cristae were lacking in the mitochondria seemed larger and the walls of the mitochondria tended to swell. In these swollen areas enlarged intramitochondrial bodies were often observed. In addition to these abnormally formed mitochondria, many osmiophilic bodies with various bizarre structures were seen (Fig. 17). These bodies were usually surrounded by a single layer of membrane and contained membranous, lamellar or granular material. Some such membranous structures resembled degenerating mitochondria; therefore at least some of the membranous material could be considered as the end-products of the degenerating mitochondria. Autophagic lysosomes, however, were rather infrequent. The cytoplasmic fibrils were markedly increased and often condensed in groups, but there was no evidence of a further condensation of fibrils as to form actual keratohyalin.

The glandular portion of the endometrium showed less striking changes, although here too no cilia or secretions were noted. The other cytoplasmic organelles were less altered in their structures. The lysosomes, particularly the large autophagic bodies, were much more numerous in areas showing a moderate leukocytic infiltration (mostly by neutrophils). Occasional lipid droplets were recognized.

The carcinoma in situ lesions had no characteristic gross appearance.

The adenocarcinoma in situ was first seen in the glandular portion of the endometrial epithelium, usually in rather discrete nodules which consisted of groups of irregular glands (Fig. 18). The glands were somewhat dilated and quite variable in their size. The lining cells were also pleomorphic, and often because of reduced cell heights and uneven cellular contours they gave the impression that they were being stretched. The cytoplasm was faintly eosinophilic. The nuclei were vesicular and large though irregular in size. Such foci of the in situ changes were quite in contrast with the loose, clear background of the surrounding stroma, which in turn differed from the much denser stroma of the dysplastic stage. This lesion developed in the glandular portions while the dysplastic appearance of the superficial epithelium continued, or in some animals revealed rather minor changes.

The cells were deprived of the ordinary secretory activities, and no cilia were seen in the in-situ cells, though some of the surface cells might show cilia. In many cells the cytoplasm was markedly dense while the nucleus was much less dense (Fig. 19). This contrast in electron density was more obvious in the low-power field. This was due in part to a general increase in the cytoplasmic density. The cells which lined the cystic spaces showed moderate pleomorphism. Most of the cytoplasmic organelles seemed to have the usual structure (Fig. 20). The mitochondria were abundant and normal in structure with regular crista formation. The rough-surfaced ergastoplasm was well-developed and showed an abundant granulation with ribosomes. Free ribosomes were also abundant and were distributed throughout the cytoplasm. A small number of smooth-membrane-bound vesicles were often seen

in some cells, which may represent smooth-surfaced ergastoplasm. The Golgi apparatus was moderately well developed. Often small vesicles were seen throughout the cytoplasm. These vesicles were definitely larger than those that were considered to represent smooth-surfaced ergastoplasm. The lysosomes were markedly increased in some cells. They were large, the largest a few microns in diameter, and had very bizarre contents of membranous structures, granules, and dense bodies. The cytoplasmic fibrils were occasionally increased, and often in areas where fibrils were accumulated free ribosomes were very sparse. Often small dense bodies, possibly lipids, were seen in some cells.

The nuclei were large. The nucleoli were also enlarged and their electron density was increased. The pars amorpha in particular had enlarged and become very prominent.

Invasive carcinoma.--The gross appearance of the chemically induced endometrial carcinomas was much less striking than that of the spontaneous carcinomas which will be described later. The induced carcinomas seldom produced the polypoid masses that were a rather common manifestation of the spontaneous tumors. The induced tumors tended to produce small nodules and to spread within the endometrial tissue (Fig. 21). In the rare instances in which the carcinomas were large enough to be recognized grossly, the tumor tissue was firm and white. An extension to the peritoneum was seldom noted. In exceptional cases pulmonary metastases were found.

Despite this great difference in their gross features, the chemically induced and the spontaneous carcinomas differed little if any in their histological picture. Both groups of carcinomas arose from

the glandular epithelium, and no definite evidence of a malignant transformation was seen in the superficial epithelium. The carcinomas were usually multifocal and composed of well-circumscribed nodules whose size and extent varied. Each tumor nodule was then formed by numerous small glands with or without lumens and by cords and sheets of cancer cells. The cell cords had many layers of cells (Fig. 22). Often small but bizarre papillary projections were noted within the tumor nodules. The small nodules were composed of smaller, more basophilic cells and the larger nodules by larger, more eosinophilic cells. In both types of nodules the cells were highly pleomorphic, with large nuclei, large nucleoli, and frequent mitoses.

In some animals the carcinomas were less differentiated. Small sheets of large basophilic cells and short strands of hyperchromatic cells with little cytoplasm lay in the deep endometrial stroma and invaded between the smooth muscle fibers of the myometrium. In one case the carcinoma was noted to involve almost the entire uterine horn with marked thickening of the wall, retention of a large amount of fluid in the cavity, and a tremendous distention and tortuosity of the uterine horn. In this rabbit tumor implantations were seen in the vagina and metastases were seen in the lungs. The original tumor of the uterus was extremely undifferentiated and was composed of spindle cells and giant cells imitating the sarcomatous pattern.

In the electron microscope, the most interesting feature of the carcinomatous endometrium was a definite morphological discrepancy between the surface epithelium and the glands. In the surface epithelium no invasion of the stroma was seen, whereas in the glandular

portions there was definite invasion of the underlying stroma.

In the superficial epithelium, as a rule, the epithelium was composed of one or more layers of columnar or cuboidal cells which varied in size and shape (Fig. 23). The columnar cells had a darker cytoplasm than the cuboidal cells. These cells showed less severe alterations than had been noted in the dysplastic surface epithelium but contained only simplified cytoplasmic structures. The luminal cell surface lacked any secretory activity. No cilia were found. There were scanty microvilli in the cuboidal cells, while in the columnar cells many more microvilli were found, which were of approximately the same length as in the cuboidal cells. In the subluminal zones of both types of cells there was a layer of moderate electron density up to 400 μ in thickness where only condensed fibrillar material was found. The lateral cell membranes were rather tightly adhered in apposition in their uppermost portions, since a number of terminal bars and desmosomes were present, in addition to some indentations of the cells. The rest of the lateral cell membranes, particularly in the basal aspects, showed moderate dissociation with widened intercellular spaces up to 2.5 μ in width. In some such distended spaces the lateral cell membranes possessed a number of microvilli, so that one could visualize apparent microcanalicular formations as a result. There were no defects in the basement membranes.

The nucleus was usually large and the nucleocytoplasmic ratio was about 1:0.5-0.6. The nuclear membrane showed marked indentations. The nucleoli became very prominent with marked enlargement of the pars amorpha and occupied a large portion of the nucleus. The nucleolonema

also became denser and often showed composite structures. Several cells showed intranuclear circular bodies as seen in the estrogen-stimulated endometrial epithelial cells.

The apical cytoplasm was relatively decreased in quantity, partly because of the enlarged nucleus. Often a moderate amount of dark fibrillar material was seen which showed a tendency to be condensed in the subluminal zone and around the outer nuclear membrane (Fig. 25). In spite of the abundance of cytoplasmic fibrils, many free ribosomes (polysomes) were distributed throughout the cytoplasm. The rough-surfaced ergastoplasm was moderately developed although its profiles occasionally were short and dilated. Membrane-bound ribosomes were abundant. The Golgi apparatus differed in its development from one cell to another. Numerous mitochondria were present in all cells seen, but in some cells they were markedly swollen. Most of the mitochondria showed the usual parallel arrangement of their cristae. Rare mitochondria, particularly small ones at the base, showed less numerous cristae. The matrix of the mitochondria was pale, and the intramitochondrial bodies were not enlarged. Lysosomes were very infrequent.

In the glandular portions of the carcinomatous endometrium active invasion of the stroma could be recognized. In many areas the cancer cells were clumped in cords or sheets and the normal glandular structure was no longer recognizable (Fig. 24). Among these cells, many intercellular microcanalicular formations were seen, and the glandular lumens, if recognizable, contained amorphous material and cellular debris.

The cells varied greatly in their appearance. Many mitoses were

present. The cells showed a wide variety of electron density. Some cells were extremely dark and showed markedly dilated, empty lumens of the ergastoplasm, in rather striking contrast to the density of the cytoplasmic ground substance. In such cells no increase in number of ribosomes was noted. In other words, this increased electron density was merely due to condensation and shrinkage of the ground substance that probably reflected the process of cellular dehydration. A few markedly electron-pale cells were seen in which often swollen mitochondria were remarkable. In some of these clear cells the areas poor in cytoplasmic organelles with only slightly increased cytoplasmic fibrils were seen; therefore this type of clear cell change was interpreted as cellular hyperhydration. In any event these extreme changes in cellular density were considered to indicate the process of cancer cell degeneration.

The nucleocytoplasmic ratio varied; in extreme cases it was down to 1:0.3, while the average was 1:0.7. The nuclear changes included a marked infolding of both outer and inner nuclear membranes without excessive separation between them and enlargement of the nucleoli. The pars amorpha of the latter showed moderate enlargement but not to the extent observed in the superficial epithelial cells, and the nucleolonema was elongated.

The cell surfaces in the carcinomatous stage were quite interesting. If lumens could be identified in the cancerous glands, a moderate number of microvilli could often be seen on the luminal cell surface. Some of these villi were short and plump, while others were long and slender. Generally the luminal cell surface was convex toward the

lumen. The lateral cell membranes were in most parts straight, and the cells were fairly well adhered to each other. As already stated, in some places there was a marked to moderate interdigitation of the lateral cell membranes (Fig. 27), often leaving small openings lined with many microvilli (microcanalicular formation). In the basal cell membrane the invasive nature of the cancerous tissue became apparent. The usual triple zone construction of the basement membranes was interrupted and the basal cytoplasmic membrane of the cancer cells directly faced the stromal collagen fibers (Fig. 28) and fibrocytes (Fig. 29) without the intervening outer dense stromal zone of the basement membrane. In these areas the carcinoma cells often showed many cytoplasmic processes protruding into the underlying stromal tissue. In some cells such changes were so advanced as to simulate microvilli, so that the cells appeared equipped with many microvilli on both luminal and lateral membranes. This suggested a possible disturbance in cellular polarity. On the other hand, there were areas where an excessive accumulation of electron-dense material in the outer zone of the basement membrane seemed to widen this layer. This may represent a defect in the polymerization and alignment of the soluble collagens, a part of which is believed to be a function of the overlying epithelial cells. In some other areas amorphous material of medium electron density, resembling mucus in the lumens of glands, was accumulated in the stroma next to cancer cells. This again may be taken as proof of an erroneous discharge of material by cancer cells. No definite secretory vesicles were seen in such cells.

The cytoplasm was slightly dark and had a moderate amount of

cytoplasmic fibrils which were obviously less abundant than in the surface epithelial cells. No condensation of these fibrils was seen in the cancer cells. Mitochondria were numerous. Many mitochondria had a rather electron-pale matrix and many cristae. Many mitochondria were markedly swollen, and in these mitochondria the cristae, themselves not swollen, seemed unable to form a bridge between the two layers of the inner wall of the mitochondrion as noted in normal cells (Fig. 26). One can only speculate whether such mitochondrial swelling depends upon the fragility of the mitochondria as a whole or upon a defect of the cristae. The ergastoplasm was well-developed. Very often its profiles appeared dilated, but membrane-bound ribosomes were numerous, as were free ribosomes. The latter were distributed rather uniformly throughout almost the entire cytoplasm. In some animals a moderate number of strongly osmiophilic bodies up to 2 μ in diameter were found. These were very dark vesicles with partly homogeneous, partly membranous contents. They were probably lysosomes.

Rat

Normal rat uterus.--The normal rat uterus has a Y shape with two uterine horns which measure about 45 to 50 mm. in length and 5 mm. in diameter and are much firmer than those of a mouse since the myometrium is well developed.

In the histological preparations the rat uterus showed a correspondingly thick, partially hyalinized myometrium. The endometrium consisted of both surface and glandular portions of epithelium and rather scanty stroma. In contrast to the mouse, the glandular

epithelium of the rat was less well developed and in general glands were seen only close to the surface epithelium (Fig. 30).

The surface epithelium was composed of a single layer of cuboidal or low columnar cells which showed dark purplish-stained cytoplasm. The cell surface was straight or slightly convex, and the immediate subluminal layer was slightly eosinophilic. The basophilia of the rest of the cytoplasm was most intense at the upper half of the apical cytoplasm just beneath the eosinophilic zone. The supranuclear area was slightly paler. The nucleus was usually basally located and often pyknotic. One or two nucleoli could be visualized. The basal portion of the surface epithelium was almost always infiltrated by a number of inflammatory cells, mostly neutrophils and some lymphocytes. During the estrous phase some cells were enlarged and their cytoplasm more osmiophilic. In others, clear vacuoles probably indicating accumulated secretion were seen. No cilia were observed.

The glandular portion of the epithelium was lined by a single layer of pale-staining cuboidal cells, and in the necks of glands, a transitional zone between surface and glandular epithelia, the cells were of an intermediate form. The cells were much shorter than the surface cells and showed little or no basophilia of their cytoplasm. The cell surface was invariably straight, and a very thin but distinct zone of eosinophilia was seen just beneath the surface. The nucleus was round and vesicular with one or two distinct nucleoli. The leukocytic infiltration was less marked than in the surface epithelium.

In the electron microscope the surface epithelial cells were short and cuboidal (Fig. 31). The cell surface facing the lumen was slightly

convex with very short, blunt microvilli. The apical portion of the lateral cell membrane was moderately tortuous. There were few, but fairly large, desmosomes. The basal portion of the lateral membrane was again tortuous; the tortuosity was more remarkable than in the apical portion.

The apical cytoplasm showed slightly electron-dense fine granular material just beneath the luminal cell surface. This opaque zone was 170-260 μ thick. Below this there was then a layer 800 μ - 1 μ thick that was rich in pinocytotic vesicles. Smaller, denser round bodies less than 100 μ in diameter, surrounded by an ill-defined single membrane, were intermingled. Tonofibrils were quite numerous. Abundant dilated rough-surfaced ergastoplasm was present. Some contained irregularly distributed, slightly electron-dense homogeneous material in the lumen. The rough-surfaced endoplasmic reticulum was not completely surrounded by ribonucleoprotein particles. Scant smooth-surfaced ergastoplasm was seen. The Golgi apparatus was rather well-developed and varied greatly in size. Some of the Golgi regions were covered with homogeneous opaque material. Closer to the nuclei, were some multivesicular bodies. Small dense bodies were also seen more frequently around the nuclei. The mitochondria were plump. They showed regular cristae. Only rarely tiny dark intramitochondrial bodies were seen.

The central cytoplasm was compressed by the relatively large nucleus, and some mitochondria, tonofibrils, rough-surfaced ergastoplasm, and more free ribosomes were present.

The basal portion of the cytoplasm contained many large lipid

droplets. Besides these some smooth-surfaced and rough-surfaced ergastoplasm and mitochondria were seen. Free ribosomes were present in moderate numbers.

The glandular epithelial cells were all cuboidal. Some of them measured up to $9.5\ \mu$ in width (Fig. 32).

The luminal cell surface was either convex or flat. There were small microvilli. Occasionally some of the cells showed elongated microvilli. The lateral cell membrane was less tortuous than that of the surface cells. The basal portion of the lateral membrane was straight and contained desmosomes.

The apical cytoplasm immediately below the luminal membrane was electron-dense and finely granular. Then beneath this zone were many pinocytotic vesicles. They were all of the same dimension as those in the surface cells. We encountered slightly more multivesicular bodies in the glandular cells. Also noted were a few dense small round membrane-surrounded bodies as seen in the surface cells. More free ribosomes were present. Most of the rough-surfaced endoplasmic reticulum was dilated. Unlike the surface cell, little electron-dense material was contained within the reticulum. The granulation of the reticulum was much less complete than in the rest of the cytoplasm. The Golgi complex was less developed in the glandular cells. The mitochondria were large and plump. The middle portion of the cytoplasm was narrow but contained some mitochondria, ergastoplasm, free ribosomes and tonofibrils. In addition to the above-mentioned structures, the basal cytoplasm occasionally contained large autophagic lysosomes measuring up to $3.5\ \mu$.

The basal portion of the cytoplasm contained much dilated rough-surfaced ergastoplasm with irregularly distributed but homogeneous electron-opaque material. Cisternae were occasionally dilated. Mitochondria were seen with the same contour as seen in the rest of the cytoplasm. A moderate number of free ribosomes were found. The matrix was finely granular. Cristae were regular. Occasionally tiny dense bodies were seen. Sometimes clear spaces 170 μ wide and lined with a single membrane were present which might represent cristae in tangential section.

Castration effect.--Castration effects on the gross appearance of the rat uterus were very obvious, the uterine horns having decreased in length to about 30 mm. and in diameter to 2-3 mm. The uterine cavities were barely recognizable on the cut surface.

The histological preparations showed a marked atrophy, particularly of the myometrium and of the endometrial stroma. The surface epithelium of the endometrium showed a marked reduction in cell size; the cells seemed much shorter due to loss of their apical cytoplasm, and even thinner (decreased cell diameter). The cells were cuboidal and much less basophilic than the normal endometrium. The nuclei were slightly dark-stained and occasionally lay transversely with their longer axis parallel to the base (Fig. 33).

An impressive change took place in the glandular epithelium. The number of glands seemed much increased per unit area of the section, although this would not necessarily mean that the total number in a uterus was increased. This was quite in contrast to the castration effect on the rabbit endometrium, where the number of glands was

markedly reduced. The glandular cells contained a minimal amount of cytoplasm, and almost the entire cell was occupied by vesicular nuclei. In some areas these glands showed cystic dilatation so that the cells were stretched or extremely flattened.

Leukocytic infiltration of the endometrium was less than that in the non-castrated animals.

With the electron microscope, tall and flat cells could be distinguished.

The tall cells had few microvilli. The lateral cytoplasmic membranes from the lumen to the basement membrane were very tortuous and had many desmosomes. The intercellular space was very narrow. There were occasional widenings between the basal lateral membranes.

The apical cytoplasm contained a large population of organelles. Membranes of rough-surfaced endoplasmic reticulum were well-developed and contained a slightly electron-dense homogeneous material. Many free ribosomes were present. The Golgi apparatus was well-developed. Lysosomes contained many vesicles and lipid droplets and measured up to 1 μ in diameter. Poorly developed smooth-surfaced ergastoplasm and many small vacuoles were present. Mitochondria were elongated to round. In the basal cytoplasm longer and narrower rough-surfaced ergastoplasm was noted. Ribosomes were scattered but less numerous than in the apical portion. There were several lipid droplets. The nucleus was large and had a large and prominent nucleolus.

The flat cells had a simpler cytoplasmic structure (Fig. 34). There were fewer mitochondria and very poorly developed rough- and smooth-surfaced ergastoplasm. Few, but large, lipid drops were present.

Occasionally there was intracytoplasmic edema with swollen mitochondria.

The glandular cells were of a single type (Fig. 35), although some of the degenerating cells had a less electron-dense cytoplasm and fewer organelles. The luminal cell surface had a few microvilli. The cytoplasm just beneath the luminal cytoplasmic membrane was moderately electron-dense. The lateral cell border was tortuous in its upper 4 μ and had numerous desmosomes. The rest of the lateral cytoplasmic membrane was straight.

The apical cytoplasm was rich in ribosomes. Some tonofibrils were present. The Golgi apparatus was moderately developed. The rough-surfaced ergastoplasm was moderately developed. There were some tubules of the smooth-surfaced ergastoplasm, some of which were distended. Some of the tubules contained a fine granular material just beneath the distinct membranes. Mitochondria were numerous. There were very few lysosomes in this part of the cytoplasm. The middle portion of the cytoplasm was narrow and contained endoplasmic reticulum and few ribosomes. The basal portion of the cytoplasm was rich in rough-surfaced endoplasmic reticulum and ribosomes with less smooth-surfaced endoplasmic reticulum. The larger lysosomes were very electron-dense and occasionally contained vacuoles. The small lysosomes were less electron-dense, had a double outer membrane, and occasionally contained smaller vesicles or membranous material. No lipid granules such as those of the surface epithelial cells were present.

The cell had a large nucleocytoplasmic ratio and distinct nucleoli. The nucleoplasm was slightly denser than that in the surface cells. The elongated nuclei were 7.5-9.5 μ in length and 3-5 μ in diameter.

The round nuclei were $6\ \mu$ in average diameter.

Estrogenic effect.--Under estrogen stimulation the uterine cavities were markedly dilated with increased secretion, and the surface epithelial cells were moderately stretched and became cuboidal or flattened. The degree of distention was more pronounced than in mice. Leukocytes in lumen and wall were remarkably abundant in rats. The endometrial stroma was thin, more fibrous, and less cellular.

After the accumulated fluid was released, the lumen shrank and the surface cells became taller and now appeared columnar with round luminal surface protruding toward the lumen. The apical cytoplasm was slightly darker than in mice. No definite pink-staining zone was discerned in the uppermost cytoplasm as was noted in mice. When secretion was not very active, cells on the surface showed some small vesicles. If secretion was active, the whole cytoplasm of pale-staining cells was altered to a rather diffuse gray, many irregular vacuoles were gathered in the apical cytoplasm, and the luminal surface became markedly irregular. Dark-staining non-secretory cells were compressed between the secreting cells. The nucleus was vesicular and the nuclear membrane was thin. The nucleolus was much bigger than that of the mouse. Later, following secretion, degeneration occurred and polymorphonuclear cells and vacuolic degeneration of the epithelial cells were increased.

The glandular crypts were few. Secretory function was very scanty. No such clear differentiation of two cell types was demonstrated as on the surface. Cells were not so tightly aggregated, and they were cuboidal, shorter than the surface cells when the lumen was not

stretched, and the cytoplasm was pale staining. The luminal cell surface was flat. The nucleus was vesicular, and the nuclear membrane was thicker and nucleoli smaller than in the surface cells.

In animals kept under prolonged estrogen treatment, cystic changes occurred very frequently in the ovaries. Then an extensive squamous metaplasia of the surface epithelium, without any sign of malignant or premalignant transformation, could be seen in the endometrium. In rare animals under such treatment, hyperplasia of the hypophysis was noticed, the largest gland measuring 6 mm. in diameter, and in the histological preparations hyperplasia of the chromophobe cells was found as the cause for this change.

In the electron microscope, the superficial epithelium consisted of tall columnar cells (Fig. 38). The luminal cell surface was convex and had many microvilli measuring 0.7μ in length. No extrusion of secretory granules was seen at or outside the luminal cell membrane. Judging from the presence of the dense bodies the process of mucous secretion probably involved a diffusion through the intact cell membrane of a soluble substance that seemed to be totally unstained by osmium. The lateral cell membrane was only slightly tortuous in the apical portion, where terminal bars were present. The upper portion of the middle segment of the lateral cell membrane was kept in apposition to that of the adjacent cells by small desmosomes. The rest of the lateral cell membrane was straight, except for the basal portion where some indentations were noted.

The cytoplasmic organelles were seen mostly in the apical cytoplasm. In comparison with those in the rabbit endometrium, the

mitochondria were much shorter and almost round. There were only a few small intramitochondrial microbodies. The ergastoplasm was well-developed and the lumens often dilated (Fig. 30). A small amount of moderately dense material was seen within the cisternae. Their membranes showed abundant granulation. In the cytoplasm were a number of free ribosomes. The smooth-surfaced agranular vesicles that were identified in moderate number probably represented the smooth-surfaced ergastoplasm. The Golgi apparatus was also well-developed. The cisternae and vesicles were abundant. In the vicinity of the luminal surface were many dense bodies as mentioned, measuring 100-500 m μ in diameter and surrounded by a single layer of membranes. They contained homogeneously medium electron-dense material within which complex substructures were seen. These included a number of tubular structures, granules, and membranous material. In addition to these dense bodies was a little dense material in club, rod, or tadpole shape.

The central portion of the cytoplasm contained many mitochondria, parts of the Golgi apparatus, and the rough-surfaced ergastoplasm. The basal portion showed some mitochondria, many ribosomes, and the rough-surfaced ergastoplasm, in addition to some parts of the Golgi apparatus.

The nucleus was elongated. The nuclear membranes showed moderate folding. The nucleoli (1-2 per nucleus) were prominent and had well-developed nucleolonema.

The glandular epithelial cells were shorter than those of the surface epithelium (Fig. 38). The cell membrane showed fewer and

shorter microvilli on the surface, and tortuosity of the lateral cell membranes was seen in the apical and basal portions as in the surface epithelial cells. The basement membrane was of the usual construction. The cytoplasmic organelles were as abundant as in the surface cells. In the apical cytoplasm were a few dense bodies of medium electron density containing various complex structures similar to those found in the surface cells. Some such structures were seen free in the lumens of the glands. The mitochondria gave the impression of being slightly larger in the glands. Golgi regions were numerous. In the basal cytoplasm of the glandular cells were well-developed ergastoplasmic channels that were interlacing and rough-surfaced. There were abundant free ribosomes and mitochondria. The nucleus had less indentation of its membranes, and the nucleoli appeared larger than those in the surface cells, with prominent nucleolonema.

Development of carcinoma.--The local application into the uterine cavity of cotton strings impregnated with 3-methylcholanthrene produced metaplasia of the rat endometrium after one month. Such a metaplastic uterus could be noted on gross examination by the lack of reaction to estrogenic stimuli, while the horn without the string showed a marked hydrometra due to increased secretory response to such stimuli. The uterine tissue became quite firm after the application of the strings, probably for the same reason.

The histological preparation showed a marked transformation of the previously columnar epithelium to squamous cell epithelium in the superficial layer. The luminal zone of such metaplastic epithelium showed a cornified layer which stained intensely blue in the thick

sections stained with toluidine blue and pyronin (Fig. 39). Several layers of cells beneath this keratinized zone consisted of elongated cells containing keratohyaline granules in the cytoplasm. This layer would correspond to the stratum granulosum of the epidermis. Further below, in the middle zone of the epithelium, several more cell layers were composed of spindle-shaped cells with vesicular nuclei. Both keratohyaline granules and nucleoli were inconspicuous in this layer. The next zone, which was 2-3 cell layers thick and located just above the basal cell zone, was characterized by rather round cells with very prominent intercellular bridges. The nuclei were again vesicular with 1-2 large nucleoli. These cells resembled the prickly cells of the skin (stratum spinosum). The basal cell layer consisted of one or two layers of cells which were slightly elongated and oriented in upright position. These cells were slightly smaller than the parabasal cells and the cytoplasm was more basophilic. The intercellular bridges were less prominent. Both nuclei and nucleoli were prominent. With the hematoxylin and eosin stain, the second and third zones of the elongated cells were less distinct since the keratohyaline granules were not so prominent as in the sections stained with toluidine blue. Parabasal cells were only recognized by the slight basophilia of their cytoplasm. The basal cells were characterized by basophilic cytoplasm and elongated nuclei. There was moderate inflammation throughout the endometrial tissue.

In the electron microscope, the luminal zone of the metaplastic endometrium showed a lamination of markedly elongated cell bodies, and desmosomes were very prominent. The cytoplasm consisted mostly of

diffusely accumulated keratin fibrils. Little or no condensation of the keratin fibrils was seen. Among these fibrils round, single-membrane-surrounded bodies were often found, which contained rather homogeneous material of medium electron density (Fig. 42). Some glycogen granules were also seen, but most of the cytoplasm lacked particulate organelles. As a rule, nuclei were not recognized in this zone.

In the middle zone of the epithelium, the cells had a typical prickle cell pattern (Fig. 40). They were spindle-shaped and were connected by numerous cytoplasmic processes (microvilli) with or without desmosomes in them (Fig. 43). There were many condensations of the keratin fibrils usually arranged parallel to the nuclear envelopes. Among the fibrils were many free ribosomes. Occasionally dense material was seen connected with keratin fibrils and free ribosomes. They resembled the keratohyalin seen in the epidermis. The other cytoplasmic organelles were scanty (Fig. 41). The mitochondria were seen only in the perinuclear regions of the cytoplasm and were arranged with their longer axis parallel to the nuclear envelopes. Their matrix was rather clear except in the vicinity of the cristae, where it was denser and appeared somewhat granular. The cristae often seemed swollen and occasionally were irregularly arranged, i.e., did not all show parallel orientation. Intramitochondrial bodies were hardly recognized. The ergastoplasm was poorly developed and only a few profiles of the ergastoplasm could be noted extending from the nuclear envelope. The Golgi apparatus was much less developed than the ergastoplasm. Lysosomes were rare. The nuclei were prominent but the nucleolar structure

was unchanged. The pars amorpha was often prominent. The structure of the cells located in the basal zone was almost similar to that of the prickle cells in the middle zone, although the former cells had somewhat smooth cell membranes with fewer bridges. The individual desmosomes appeared larger and the intercellular spaces were often wider than in the above layer. At the basement membrane, the formation of hemidesmosomes became quite obvious. The cytoplasm showed reduction in the total amount of keratin fibrils, although they now tended to condense in bundles and so became conspicuous. Thus the mitochondria were pushed aside, closer to the nucleus. Some of the mitochondria showed moderate swelling. The ergastoplasm was abundant in the basal cells and many short profiles of the organelle with moderate ribosomal granulation were seen. No cisternae were dilated. Also the free ribosomes seemed increased.

The dysplastic stage was observed from two months after the insertion of the strings. The thick plastic section showed little or no keratinization--definitely less than seen in metaplasia in many cases (Fig. 44). In general, the cells were larger than those of the metaplastic stage and the cellular borders stained more intensely than the rest of the cytoplasm, which was very pale. The nuclei were enlarged but less so than in the preceding stage. The keratohyaline granules, so abundant in metaplasia, seemed to have diminished in many cells located in the subluminal zone. In other words, the typical appearance of the stratum granulosum was not clear in this stage, and most of the cells assumed a prickle cell appearance. The basal cell zone had smaller cells having dark-staining cytoplasm and oriented in vertical

position. The intercellular space was widened in the basal portions of the epithelium, and the intercellular bridges were very prominent.

The vesicular nuclei and the nucleoli were strikingly enlarged although the size of the nuclei varied somewhat. In some of the dysplastic epithelium cellular dissociation was marked, more than a half of the epithelium having torn off and fallen into the lumen leaving only one or two layers of the basal cells in place. In general, there was no keratinization in such loosely constructed epithelium, which probably indicated lack of desmosomes.

The electron microscope revealed few structural differences between the metaplastic and dysplastic stages beyond those just described at light microscopy level, since the alterations were in cellular architecture of the epithelium and the outlines of the cells rather than changes in the organelles within individual cells.

In general, only one or two cell layers in the luminal zone were keratinized. Most of the prickly cells, which constituted the main fraction of the epithelium, showed a moderate amount of keratin fibrils (Fig. 45). Keratohyaline granules and the associated structures were present in some cells in a small quantity. Besides the typical keratohyaline granules, membrane-bound bodies 100 μ in diameter and containing dark fibrillar to granular material were present. These granules may correspond to the membrane-bound granules present in certain squamous epithelial cells. Some structures within the membranes, about 10 μ in diameter, resembled microtubules. Occasional dense granules 50 μ in diameter and a moderate number of mitochondria were also present in the cytoplasm. Occasionally less electron-dense cells were

seen. In these cells the keratin fibrils and free ribosomes were dispersed sparsely, and the mitochondria and the ergastoplasmic lumens were dilated. The appearance of these cells suggested a difference in the state of cellular hydration. Many cells showed a moderate to marked swelling of the mitochondria (Fig. 46). These mitochondria contained a very electron-transparent matrix, but the large intramitochondrial bodies present in the dysplastic stage in mice were not observed. The ergastoplasm consisted of a few short profiles of the rough-surfaced type. Some of the cisternae were dilated, but the majority were not. Free ribosomes were fairly abundant. The Golgi apparatus was totally inconspicuous. The basal cells differed slightly from the prickle cells. The former cells contained less keratin fibrils, were bound to the basement membrane with many hemidesmosomes, the ergastoplasmic channels were better developed, and the mitochondria were also more numerous. Keratohyaline and membrane-bound granules were seldom present. Despite the significant nuclear changes observed in light microscopy, the ultramicroscopic changes in the nuclei were rather subtle.

The carcinoma in situ stage was recognized by the marked cellular abnormality. The superficial keratinization was thicker in some areas than others. There was definite evidence of disturbed cellular polarity (Fig. 47). In the hematoxylin-eosin preparations the upper half of the epithelium was composed of many large eosinophilic cells. The nuclei were large and pleomorphic; huge nucleoli were present. Occasionally in the subluminal zone several keratohyalin-containing cells were present. The basal cells were hyperplastic as shown by an

increased number of cell layers. They had basophilic cytoplasm and large nuclei and nucleoli.

Under low-power view in the electron microscope, the density of the cytoplasm was considerably increased so that in some cells the cytoplasm appeared denser than the nucleoplasm (Fig. 48). The ultrastructural details were little altered from the preceding stage.

In the luminal zone the process of keratinization appeared slightly different. In the metaplastic stage there were abundant keratin fibrils and some dense particles in this zone. In the carcinoma in situ stage there were in addition several clear spaces which might have been derived from the pre-existing lumens or cisternae of the ergastoplasm. A very few mitochondria were seen, which could be recognized with some difficulty since they were undergoing disruptions. No lysosomes were identified. There were some nuclei in this zone, which showed ill-defined nuclear envelopes and moderately dense karyoplasm. The ergastoplasmic lumen immediately surrounding the nucleus seemed dilated. Whether such observations indicated a rapid process of keratinization or represented parakeratosis was not determined.

The main portion (middle layers) of the epithelium showed many prickly cells. The cell was not elongated, and there were numerous cellular processes contributing to intercellular bridge formation in which many prominent desmosomes were seen. Keratin fibrils were very abundant and in many bundles in the cytoplasm. The secondary particles related to keratinization were markedly increased in number as compared to the dysplastic cells (Fig. 49). As a rule they were found in the

peripheral portions of the cytoplasm, particularly between keratin fibrils. Those most frequently encountered were the previously mentioned membrane-bound granules. Some of them had no definite outer coverings. Some keratohyaline granules were also present. Clumps of glycogen granules and single glycogen granules were observed. The rough-surfaced ergastoplasm was well developed, its channels interlaced throughout almost the entire cytoplasm. Mitochondria were also abundant and had a pale matrix and tiny intramitochondrial microbodies. Some mitochondria were swollen but the usual number of cristae were present. Lysosomes were not observed.

The basal cells in glutaraldehyde-fixed preparations showed a marked indentation (Fig. 51). This might indicate that the cytoplasmic membranes of the basal cells differed somewhat and were less resistant to osmotic pressure. The basement membranes in the in-situ phase did not show any disruptions as seen in the invasive stage but were markedly tortuous.

The nucleus in this stage was usually large and contained large nucleoli. The pars amorpha of the nucleoli was dense, and karyosomal condensation of the chromatin in proximity to the inner nuclear membrane was noticeable. Even within the nucleus the karyosomes were condensed, although to a lesser degree. Many dense particles measuring 120-350 μ were also seen (Fig. 50). These were probably ribonucleoprotein but were definitely not connected with the nucleoli. They differed also from parachromatin granules since there were no halos around them.

Invasive carcinoma.--Upon gross examination, the carcinoma

presented itself as a large mass originating from one of the uterine horns (Fig. 52). These masses averaged 3 cm. in diameter; the largest measured 5 cm. Most of the involved uterine horn was embedded in the tumor mass and only a small distal portion of the uterine horn and the cervix could be identified on the surface of the mass. There were many adhesions between the mass and loops of intestine. The serosal surface covering the mass had no tumor nodules but showed severe edema, probably due to compression and obstruction of the lymphatic channels. On cut surface the central portion of the mass consisted of highly malodorous necrotic tissue surrounded by a thick fibrotic wall (Fig. 53). The carcinogen-impregnated string was found either in its entirety or in fragments within the necrotic material. No tumor metastases were found.

In the histological preparations, the majority of the carcinomas were well-differentiated squamous cell carcinomas (Fig. 54). The necrotic cavity seen grossly contained much cancer cell debris and many leukocytes, and the wall in part was lined by nonspecific fibrous tissue, in part by squamous cells. Considerable fibrosis was seen. The carcinomatous nodules were rather well delineated. The luminal portion of the cancer tissue showed a zone of keratinization of varying thickness. The rest of the malignant epithelium was very rich in cells which showed a total loss of polarity. The cancer cells were usually fairly large and had no regular orientation. The cells directly below the keratin layer had rather swollen, clear cytoplasm in which few keratohyaline granules could be seen. The middle zone of the epithelium was formed by irregularly piled-up large cells with

eosinophilic cytoplasm. Nuclei were also large but markedly dissimilar in size. The basal cell zone often had lost the usual basophilia of the cytoplasm, and the vertical arrangement of the cells was frequently disturbed (Fig. 55). The cells were large and had large nuclei and huge nucleoli. Many mitoses were seen. These basally located cells resembled in some respects the prickle cells or parabasal cells seen in metaplasia.

In the thick sections fairly abundant keratohyaline granules were seen immediately beneath the keratinized zone, which was composed of large prickle cells.

The carcinomas produced by the insertion of strings treated with the paraffin-polyvinyl resin mixture without carcinogen were all squamous cell carcinomas which were indistinguishable from the carcinogen-induced tumors.

In study with the electron microscope, the rat carcinomas were of typical squamous cell type but showed more keratinization than the squamous cell carcinomas induced in mice. The cell membrane showed the typical indentation of a squamous cell. Numerous microvilli were found, with or without desmosomes. The majority of the cells composing the carcinomatous tissue resembled, with respect to organization of the keratin fibrils and the structures associated with them, the prickle cells seen in the stage of squamous metaplasia (Fig. 56).

The luminal (upper) portion of the carcinomatous epithelium consisted of a few layers of cornified cells. The cytoplasm of the uppermost cells had been almost completely replaced by diffusely distributed keratin fibrils. The cellular membranes were dense and thick throughout

the cell surface, reaching a thickness of 10-20 μ in some areas where the cell membrane had a double layer. Adhesion plates were often present between apposing cell membranes. In the first several superficial layers of cancer tissue, each of which measured approximately 1 μ in thickness, no mitochondria or other cytoplasmic organelles were identified.

The subluminal cells were elongated, as in the stratum granulosum of the epidermis. They had a large amount of keratin fibrils and very prominent desmosomes. Their mitochondria were obviously reduced in number and some were undergoing disruption. Besides rare, small accumulations of free ribosomes, many small bodies were seen among the keratin fibrils. Round bodies of medium electron density and measuring 120-150 μ in diameter were found surrounded by some keratin fibrils. The inner structure was homogeneous, and no fibrils were noted within these bodies. The presence of a double layer of membranes was suggested in some of these bodies. In addition, there were intensely osmophilic larger bodies with a diameter of 200-400 μ and a rare small one (about 70 μ). These structures were not membrane-bound but also showed a connection with keratin fibrils. Frequently some of the keratin fibrils could be traced into the interior of these bodies. The centers of these bodies were homogeneously electron-dense, and the peripheries were studded with ribosomes. They were therefore identical to the so-called keratohyalin observed by many authors in the epidermis of humans and other species.

The middle layer of the carcinomatous epithelium consisted of many squamous cells in a typical spinous form (prickle cells) and a few still

slightly flattened. The ultrastructure of the two types was almost identical. Bundles of keratin fibrils were abundant, were located mostly in the peripheral portions of the cell, and were aligned in an annular pattern as though to surround the nucleus at some distance. Abundant mitochondria and free ribosomes, mostly in the central or perinuclear portions of the cytoplasm, were surrounded by whirls of keratin fibril bundles (Fig. 58). The mitochondria were usually clumped and were short and plump, an average-sized mitochondrion measuring $1\ \mu$ in length and $700\ m\mu$ in diameter. A few free ribosomes were seen even among the keratin fibrils in the peripheral cytoplasm. The ergastoplasm, however, was scanty. Where their membranes could be visualized, they had only a small number of ribosomes, in contrast to the abundance of free ribosomes. The ergastoplasmic cisternae were empty and not dilated. The outer nuclear envelope, which constitutes a part of the rough-surfaced ergastoplasmic system, was also poorly granulated. Golgi apparatus was hardly recognizable. Lysosomes were absent in the majority of the cells. The nucleus showed moderate infolding of the inner nuclear membrane, but karyosomal condensation was minimal. The karyoplasm was uniform and of medium electron density and contained very prominent nucleoli. The nucleolonema was very well developed. In some cells the nuclei contained many dark granules which were associated neither with nucleoli nor with halo-like clear zones (as seen in the case of the perichromatin granules) (Fig. 57). These bodies seemed to be tightly packed granular material, probably ribonucleoprotein, and measured $95-150\ m\mu$ in diameter. The nucleocytoplasmic ratio of these malignant cells was about 1:0.6-0.7.

The basally located cancer cells differed slightly from the basal cells in the stage of dysplasia. In the electron microscope, the basal and prickle cells could hardly be differentiated except in a few respects: In the former the intercellular bridge formation was less remarkable, and microvilli and desmosomes were less numerous. The amount of keratin fibers was definitely reduced while the amount of free ribosomes and the number of mitochondria were increased. The rough-surfaced ergastoplasm was better developed although the granulation of its membranes was not very abundant. The mitochondria had the electron-pale matrix seen in the rest of the cancerous epithelium, and very faint granularity was seen. These granules had about the size of a ribosome and differed definitely in size and electron density from the usual intramitochondrial microbodies. The formation of hemidesmosomes on the basement membrane was very prominent. Parts of the cytoplasmic membrane and the corresponding outer dense zone of the stroma were thickened. The basal cytoplasmic membrane reached a thickness of 30 μ , and there was a second thin electron-dense layer about 10-15 μ apart from the cell membrane within the electron-transparent zone of the basement membrane. The nuclei of the basal cells showed less infolding of the nuclear envelopes. The laryoplasm was still homogeneous. The nucleoli seemed somewhat larger than those in the prickle cells, and the nucleolonema was very prominent.

Mouse

Normal mouse uterus.--The normal mouse uterus was Y-shaped with two horns which measured about 20 mm. in length and 2 mm. in width. In histological sections the uterine cavity of the diestrous uterus

was lined by superficial epithelium and many non-branching glands extended into the stroma (Fig. 59). The surface epithelium of the endometrium consisted of a single layer of non-ciliated columnar cells which stained much darker than the glandular cells. The cell surface was straight and showed very distinct eosinophilia at the surface. Immediately below this zone the apical cytoplasm was pale, but most of the apical cytoplasm stained very darkly with some basophilic tint. The supranuclear zone again stained faintly while the infranuclear cytoplasm was eosinophilic. The nucleus was elongated and darkly stained. Only small nucleoli were found. The glandular epithelium consisted of a single layer of cuboidal cells having minimal cytoplasm. Their nuclei were round or even flat with the longer axis lying perpendicular to the basement membrane. The nuclei were vesicular and one or two distinct nucleoli were present. The underlying endometrial stroma was rich in fibrocytes and contained many leukocytes.

In the electron microscope, the surface cells were all non-ciliated. The luminal surfaces of the cells were slightly convex and had a few short microvilli (Fig. 60). No secretory activity was noted in the epithelium although some electron-dense material was attached extracellularly.

The lateral cell membranes of adjacent cells were closely connected. In the apical portion of the cells this was by means of moderately winding membranes with some terminal bars. In the middle portion, there was a narrow intercellular space between straight cell membranes having a few small desmosomes. In the basal portion a moderate invagination

of the lateral cell membrane was again seen. No desmosomes were present in this area.

In the apical cytoplasm the subluminal zone of about 100 μ there was some condensation of fibrillar material. Below this zone free ribosomes (polysomes) were less evenly and densely distributed than in the basal cytoplasm. The rough-surfaced ergastoplasm was poorly developed. The mitochondria were small. The cristae were very uniform, and the matrix was very electron-dense. The mitochondrial bodies were very small. The Golgi apparatus was adequately developed. The cisternae were moderately electron-dense. Many electron-dense bodies were present in the apical cytoplasm, with diameters up to 1.2 μ , bound by a single membrane. Some of them contained homogeneous material. The contents of others was very complex with accumulation of small membranous fragments, vesicles, and granules embedded in a dense background. The latter may represent lysosomes.

The middle portion of the cytoplasm formed narrow strips between the nucleus and the lateral cell membranes. Some mitochondria, rough-surfaced ergastoplasm, Golgi apparatus and free ribosomes were present.

The basal cytoplasm was characterized by the presence of numerous stellate lipid droplets about 450 μ in diameter (Fig. 61). The ground substance of the cytoplasm at the base was slightly more electron-dense, and free ribosomes were more densely distributed than at the apex. Rough-surfaced ergastoplasm seemed more developed in this portion. The mitochondria were as in the apical portions. The Golgi apparatus was scarce. Lysosome-like bodies were smaller and less frequent.

The glandular epithelium contained cells almost identical with

those in the surface epithelium (Fig. 62). The cells were slightly shorter. The luminal surface was usually flat with a few microvilli. The lateral cell membrane for the most part was straight, showing minimal tortuosity only in its basal portion.

The subluminal 300 m μ layer of the cytoplasm was moderately electron-dense and contained only a few tiny, empty vesicles which probably represented pinocytotic vesicles. The rest of the apical cytoplasm had a well-developed Golgi apparatus and narrow but moderately rich rough-surfaced ergastoplasm (Fig. 63). There was moderate granulation of the ergastoplasmic membranes. The free ribosomes seemed more abundant than in the apical cytoplasm. The structure of the mitochondria was similar to that in the superficial epithelial cells. The dense bodies described in the surface cells were less frequent in the glandular cells; however, they had a more pleomorphic appearance. Rather homogeneous dense bodies bounded by a single membrane, dense crescent structures connected with medium electron-dense material, and membrane- and vesicle-containing dense bodies were present (Fig. 64). Also rare lipid droplets were observed close to the basal cytoplasm, occasionally in close contact with mitochondria. These droplets were less numerous than in the surface epithelial cells. Some glandular cells lacked such lipid droplets.

The nuclei of the glandular epithelial cells showed more condensation of the chromatin material (karyosomal condensation), and the nucleoli were more prominent, particularly in the nucleolonema. The indentation of the nuclear membranes was more pronounced in the glandular epithelium than in the surface cells.

Castration effect.---The mouse uterus showed marked atrophy after castration. Both uterine horns became almost thread-thin; they were also shortened. The histological sections revealed marked atrophy of both epithelium and stroma; in the latter the reduction in the cell population was remarkable (Fig. 65). The cellular changes were most pronounced in the surface epithelium. These cells became short due to loss of the apical cytoplasm. The cell surface was still straight and darkly stained, but in the rest of the cytoplasm zones with varying staining characteristics as seen in the normal diestrous cell were not distinguishable. The basal cytoplasm was also reduced in quantity. The nuclei were still elongated and showed more vesicular staining than in the diestrous stage. The glands seemed close together, probably due to reduction of the stroma. The appearance of individual glandular cells differed little from that seen in normal diestrus.

In the electron microscope, the surface epithelium consisted of a single type of non-ciliated epithelial cells. The luminal surface was minimally convex and had small, scanty microvilli.

The lateral cytoplasmic membrane was similar to that of the diestrous cells.

The cytoplasm was rich in fine structures. The variety of lysosomes was very striking. The apical cytoplasm contained many mitochondria. The rough-surfaced ergastoplasm was less developed than the diestrous cells and not completely surrounded by ribosomes. A few vesicles of the smooth-surfaced ergastoplasm were present and some of them were swollen. The Golgi apparatus was well-developed (Fig. 66). A few cells, particularly those at the neck of glands, contained a

few small round black bodies similar to secretory granules in the subluminal areas. Multivesicular bodies were present only in the apical part of the cytoplasm. Large, darkly stained autophagic vacuoles were rare. Many free ribosomes were also present. The middle portion of cytoplasm contained some mitochondria, both rough- and smooth-surfaced ergastoplasm, and ribosomes. Scanty Golgi apparatus was seen. In the basal portion the cytoplasm contained many mitochondria and lipid droplets, which were often in close connection (Fig. 67). Some vesicles of the rough- and smooth-surfaced ergastoplasm were present. Moderately large lysosomes, particularly autophagic vacuoles were present.

The nuclei were rather large with a high nucleocytoplasmic ratio.

The glandular epithelium was composed of a single type of cell which closely resembled the surface cells (Fig. 68).

The luminal surface was similar to that of the surface epithelium. So was the lateral cell border except for the straight basal portion.

The apical portion of the cytoplasm was much simpler than that of the surface cells and no secretory granules were found. There were many ribosomes and more smooth-surfaced ergastoplasm. Little rough-surfaced endoplasmic reticulum was seen, and the coating by ribosomes was not quite complete. Some microvesicular bodies were seen, as well as rather well-developed Golgi apparatus. The middle portion of the cytoplasm contained a moderate number of mitochondria with very rare intramitochondrial bodies. The basal portion of the cytoplasm did not contain lipid droplets. Long rough-surfaced endoplasmic reticulum was present. Large lysosomes were more prominent than in the surface cells.

The nucleoli were prominent but not very large.

Estrogenic effect.--The estrogen stimulation by means of subcutaneous or intramuscular injections of diethylstilbestrol seemed to exert its effects mostly between the second and fourth days after injection.

In the mouse, estrogen treatment produced a remarkable hydrometra due to hypersecretion of the endometrium. The uterine wall became semitransparent (Fig. 69). In the spontaneous estrus the uterine enlargement was less. In a few of the treated animals a marked hypertrophy of the muscular tissue of the bladder neck and a marked distention of the urinary bladder developed. In extreme cases the uterus measured up to 40 mm. in length and 15 mm. in diameter. In contrast to rats, superimposed infection or cystic changes of the ovaries were rare. In histological preparations, the lumens contained debris of epithelial cells and serous fluid. The surface epithelium was hypercellular and presented a crowded appearance. In the upper portions of the uterine horn the epithelial cells appeared cuboidal, apparently due to increase in hydrostatic pressure caused by retention of secretion in the uterine cavities. In the lower segment of the horn the surface cells were still columnar in shape. Attached to the cell surface was a layer of mucus. The cell surface was eosinophilic. Below this zone the main portions of the apical cytoplasm were less basophilic than in the diestrous mice, but in some instances it appeared eosinophilic. The nuclei were slightly more vesicular and elongated. Often both apical and basal cytoplasm showed vacuolization. Occasionally the intercellular space was distended with serous fluid. The glandular

epithelium was lined by a single layer of columnar to cuboidal cells. These cells were taller than those in the diestrus (Fig. 70). The majority of the cells presented pale-staining cytoplasm, and their cell surface protruded irregularly into the lumen. A small number of cells showed dark cytoplasm and contained homogeneously eosinophilic cytoplasm, while the pale cells had a faintly stained supranuclear region. The nuclei in both types of cells were relatively small and occupied the central portion of the cells. Some dark cells had pyknotic nuclei. The nucleoli were also very small.

In the electron microscope, the presence of dark and pale cells was less distinct than in the sections stained with hematoxylin-eosin. In some areas, particularly in the superficial epithelium, many cells had a rather swollen, slightly electron-dense apical cytoplasm with few cytoplasmic organelles, presumably because of hyperhydration of the cytoplasm. In the glandular portion, however, most of the cells contained many secretory products and cytoplasmic organelles. In the superficial epithelium the cells were very tall. The luminal cell membranes lacked cilia. They are convex and covered with many microvilli. The apical cytoplasm had a moderate number of membrane-bound dense granules which were very pleomorphic. These bodies were considered to be intracellular forms of secretion. The mucus did not stain with osmium, and the actual process of secretion could not be visualized. The majority of such dense bodies measured 0.8-1.4 μ in diameter and were surrounded by a single layer of membrane to which a few electron-dense granules were occasionally attached. In some dense bodies, the presence of a limiting membrane was obscure. These dense bodies

contained tiny vesicles or entangled lamellar structures, all gathered in a bizarre pattern and embedded in a rather homogeneous substance of medium electron density (Fig. 72).

Mitochondria were long and slender. The matrix was slightly electron-dense and the intramitochondrial bodies were often enlarged. One week after the last injection of diethylstilbestrol, when the secretion provoked by the injection had ceased, the enlargement of the intramitochondrial bodies was very remarkable; however, these granules were not as large as the intramitochondrial bodies present in carcinogen-induced dysplasia of the epithelium. The cristae were transversely arranged, perpendicular to the long axis. The ergastoplasm was mostly of the rough-surfaced type, and in the apical cytoplasm the cisternae were dilated. There were numerous free ribosomes and polysomes. Many small smooth-membraned vesicles, larger than the vesicles of the Golgi apparatus, were seen in the apical cytoplasm. These vesicles probably were parts of the smooth-surfaced ergastoplasm. The development of the Golgi apparatus was less than that in the glandular epithelial cell. The cisternae of the Golgi apparatus were narrow. Few cytoplasmic fibrils were present.

The glandular epithelial cells had slightly different ultrastructural characteristics compared with those of the surface epithelium. There were numerous small electron-dense granules in addition to the larger complex dense bodies that were also present in the surface cells (Fig. 71). The smaller granules were quite different from the latter bodies in that their matrix was a homogeneous material. These granules measured from 100-400 m μ in diameter. The basal cytoplasm contained no

basal lipid droplets. The microvilli on the luminal surface of the cells were larger and more numerous than in the surface cells. Both the Golgi apparatus and the smooth-surfaced ergastoplasm were more highly developed. The mitochondria were similar in appearance.

Development of carcinoma.--The mouse endometrium exhibited squamous metaplasia two weeks after insertion of the 3-methylcholanthrene-impregnated cotton strings (Fig. 73). The process of metaplasia appeared to progress gradually from the surface into the deeper portions of the glands. In the electron micrographs, the glandular cells in early metaplasia showed a moderate secretory response to estrogenic stimuli (Fig. 75), but in the later stage such response to the estrogens disappeared (Fig. 76). The necks of the glands showed a transitional appearance between the metaplastic surface cells and the typical glandular cells.

The carcinogen-treated uterine horn was dilated by insertion of the string. There was little, if any, secretion, even after estrogen administration, while the untreated horn contained a significant amount of secretion. The carcinogen-treated horn showed an increased tissue consistency and slight inflammation in the cavity of the uterus, although the infection was not as severe as in the rat uteri with carcinogen-impregnated strings.

The histological preparations showed a thin squamous epithelium at the endometrial surface composed of several layers of large cells connected by numerous intercellular bridges. In the later stage of metaplasia the epithelium appeared thickened and there was a thin layer of keratinization in the uppermost portion of the epithelium (Fig. 74).

Immediately below this keratinized zone were several layers of cells transversely oriented. These cells were elongated and their borders stained intensely with toluidine blue, in contrast to a faint staining reaction of the cytoplasm. The cells were closely packed and few intercellular bridges were distinguishable. Few keratohyaline granules were seen. This layer in its cellular appearance probably corresponds to the stratum granulosum of the epidermis. The next lower layer was composed of larger cells with a typical prickle cell appearance. The cells were not elongated and had numerous intercellular bridges. The nuclei were enlarged and some nucleoli were very large. The basal cell zone was formed by smaller, slightly elongated cells facing the basement membrane in a perpendicular direction. These cells had very little cytoplasm. Their vesicular nuclei contained two distinct nucleoli. A number of leukocytes were present in the stroma and invaded the surface epithelium. In the deeper portion of the glands the epithelium retained its simple columnar or cuboidal construction. A transition stage between the surface and glandular epithelia was present in the uppermost portion of the epithelium.

In the electron microscope, the superficial epithelium showed a decreased number of cytoplasmic organelles and an increase in keratin fibrils; however, the keratinization was never as extensive as observed in metaplastic endometrium of rats (Fig. 77). The numbers of keratinized cells and of keratohyaline granules in the upper portion of the mouse epithelium were quite small. The subcellular structures were fairly well preserved even in the cells lying at the uppermost layer of the mouse epithelium (Fig. 78). There were only minor

differences, such as a slightly increased amount of keratin fibrils, between the cells at the luminal border and those in the intermediate layer of the epithelium.

The intermediate zone of the epithelium was composed of typical prickly cells. The cells were spindle-shaped or elliptical. There were numerous closely apposed indentations of the cell membranes, such that a very narrow intercellular space was present between the cells. In some areas this intercellular space widened and the cell membranes were connected by many desmosomes. The cytoplasm contained abundant keratin fibrils with no tendency to conglomerate or condense. The fibrils were diffusely distributed throughout the entire cytoplasm and were connected with the desmosomes. Other cytoplasmic organelles were markedly reduced in number as compared with the normal cell. There were a few mitochondria and very little rough-surfaced ergastoplasm. The mitochondria were round or short. Their matrix was moderately electron-dense and had a dark, granular appearance. The intramitochondrial bodies were small. The profiles of the rough-surfaced ergastoplasm were short and dilated. A moderately homogeneous electron-dense material was present within the cisternae. The membranes had many ribosomes. The Golgi apparatus was poorly developed. Free ribosomes were extremely scarce. Keratohyaline granules were very rare. Occasional accumulations of glycogen granules were present.

The basal cells were much smaller than the cells of the intermediate zone. The cytoplasm contained only a small amount of keratin fibrils (Fig. 79), which were less diffusely distributed in the cytoplasm of these cells than in those of the upper layers of the epithelium.

The fibrils were generally in bundles located about half way between the cell and nuclear membranes. Polysomes were numerous and distributed throughout the cytoplasm. Both mitochondria and rough-surfaced ergastoplasm were abundant. The mitochondria had a slightly electron-dense matrix and clearly outlined cristae. They contained intramitochondrial bodies that were somewhat enlarged but did not reach the size of the bodies present in some of the estrogen-treated mice. The rough ergastoplasmic membranes were more numerous than that in the cells of the intermediate zone. The cisternae were less dense. Lysosomes were absent. The Golgi apparatus appeared to be larger than that in the cell of the upper portions of the epithelium. Just above the basement membrane many basal lipid droplets were present, as also seen in the normal cells. There were many desmosomes, which were moderately larger than the hemidesmosomes present at the base of the cells.

The glandular epithelium in this stage showed numerous secretory granules in the apical cytoplasm of the cells (Fig. 75). However, these granules were considerably decreased or absent from the cells in late metaplasia (Fig. 76). The cytoplasmic organelles (rough-surfaced ergastoplasm, Golgi apparatus, mitochondria, and polysomes) were very abundant.

In the dysplastic stage, the epithelium was moderately thickened and the cells in general were enlarged. Keratinization was present only in limited areas (Fig. 81). The subluminal zone of the epithelium, composed of spindle-shaped cells without keratohyaline granules, also was not prominent. Thus the majority of the constituents of the epithelium were typical prickly cells. The prickly cells had a densely

stained cytoplasm and vesicular nuclei. The nucleoli were large. The basal cell zone was crowded with smaller cells which were aligned on the basement membrane in upright position (Fig. 80). Numerous acanthotic downward projections of the basal layer resulted in a wavy appearance of the basement membrane. The nuclei of the basal cells were vesicular and the nucleoli were very prominent. The polarity of the cellular arrangement was not disturbed. A moderate inflammatory reaction persisted through this dysplastic stage. While the surface epithelium underwent dysplastic change, the glandular epithelium showed only occasional cystic dilatation of the glands, probably due to obstruction of the stoma by the metaplastic surface epithelium.

The ultrastructural changes during the dysplastic stage were the most outstanding ones observed in the entire course of the chemical carcinogenesis. The superficial epithelial cells had a greater reduction in their cytoplasmic organelles than in the metaplastic cell. The cytoplasm, particularly near the nuclei, contained few structural elements. The keratin fibrils were separate and fragmented. In addition few, small mitochondria and many round bizarre inclusions composed of membranous and/or granular material were present (Fig. 83). The inclusions were about $1\ \mu$ in diameter. Because of their proximity to recognizable mitochondria, and because of the occasional presence of the dark round bodies seen in some mitochondria, some of these bizarre structures were presumed to be mitochondria also. The rough-surfaced ergastoplasm was poorly developed. The Golgi apparatus was seldom recognized. If seen, the cisternae were filled with some electron-dense material. Free ribosomes were present in small clusters and

their total amount over all cells seemed definitely reduced.

The middle layer of the epithelium, as in the metaplastic stage, was composed of a number of prickle cells. The general contour of the cells was round. The cell membrane had multiple processes which were parts of intercellular bridges. Many of them were associated with desmosomes. The cytoplasm had many mitochondria and a well-developed rough-surfaced ergastoplasm. Often large accumulations of glycogen granules were present and in close connection with mitochondria.

A large number of mitochondria had huge inclusion bodies (Fig. 84). The mitochondrial membranes were often distended by these bodies. These bodies were round and had a homogeneous electron-dense matrix. Some were very osmiophilic, while others (usually the smaller ones) had only medium osmiophilia (Fig. 85). They also varied in size; their diameter was from 80 to 450 μ . The smallest ones were between cristae with no apparent direct contact, while the larger ones were completely surrounded by cristae.

The prickle cells often had small granules which were considered to be keratohyaline granules in their cytoplasm. The granules were round, elliptical, or tadpole-shaped, and measured 300-400 μ in diameter. They consisted of very osmiophilic material, often denser than the aforementioned intramitochondrial bodies. No definite membrane surrounded these bodies. In some cells smaller, single membrane bound granules were present that did not exceed 200 μ in diameter and were fewer than the dark keratohyaline granules. The nucleus and nucleolus showed no changes from those of the metaplastic cells.

The basal cells had an appearance similar to the prickle cells,

and the subtle differences between these two types were the same as those observed in the metaplastic cells. Only a few of the cells had enlarged intramitochondrial bodies and even these were much smaller than those seen in the prickle cell layer.

Squamous cell carcinoma in situ of the mouse endometrium was observed as early as two months following initiation of the experiments. The epithelium was not always as thick as in dysplasia, but the cellular polarity was severely deranged (Fig. 87). Keratinization was not as marked as in metaplasia. The number of keratohyaline granules visible in the histological sections was limited. The epithelium was composed of large cells in which the staining characteristics of the cytoplasm were variable. In some cells the cytoplasm stained extremely darkly, particularly with toluidine blue. Often the cells were too closely packed to allow visualization of intercellular bridges. The morphological differences between the cells in the intermediate zone and those in the basal zone were minimal, since most of the cells in these regions were of a basal cell type (Fig. 86).

Similar observations were also obtained from the electron microscopic examination. The majority of the epithelial cells simulated the basal cells, especially in the appearance of the cytoplasmic structures (Fig. 88).

The transition from the dysplastic stage to carcinoma in situ was associated with the disappearance of the huge intramitochondrial bodies (Fig. 89).

The surface zone of the epithelium had several cornified layers of cells that contained many keratin fibrils. Among the dispersed

keratin fibrils numerous vesicles were often present. Some of the vesicles were rough-surfaced ergastoplasm.

Both the middle and basal layers of the epithelium were formed by round or elliptical cells which were connected to one another by desmosomes. Often the cells were so closely interlocked that intercellular bridges were indistinguishable under lower power magnification. The basal layer of the epithelium had many hemidesmosomes as well as numerous cytoplasmic processes just above the basement membrane. No destruction of the basement membrane was observed. Certain basally located cells extended toward the stroma but were always lined with the basement membrane. This type of organization produced a ragged appearance of the basement membrane.

Fewer keratin fibrils were present in most of the cells as compared with the dysplastic cells. Most of the fibrils were in bundles. Very few mitochondria had enlarged intramitochondrial bodies, but the increase in size was very small compared with that in dysplasia. As in the basal cells, the rough-surfaced ergastoplasm was moderately well-developed. The Golgi apparatus was equally well-developed. Numerous free ribosomes were distributed throughout the cytoplasm. There was often an increase in lysosomes, particularly in large autophagic bodies. The nucleus showed increased folds of the nuclear membranes, and the chromatin showed fairly large areas of condensation (karyosomes) near the inner nuclear membrane. The nucleoli were similarly enlarged, and both nucleolonema and pars amorpha were hyperplastic.

Invasive carcinoma.--The carcinomas that developed in the mouse endometrium were large, up to 2-3 cm. in diameter. The center of the

tumor was necrotic and the wall was composed of firm fibrotic tissue. The tumors often adhered to loops of the intestine and to the abdominal wall. Metastases were not present. In many instances the uterine cervixes were not involved and were on the surface of the tumor mass. The uterine horn into which the carcinogen-impregnated string had been inserted was destroyed beyond gross recognition. The majority of the tumors were well-differentiated squamous cell carcinomas originating from the surface epithelium (Fig. 90). Some areas of the surface epithelium showed extensive malignant transformation although evidence of invasion of the underlying stroma was absent. The cancerous squamous epithelium had only a minimal degree of keratinization or of parakeratosis at the surface, and keratin pearls were rather infrequent. Instead, the superficial cancer cells had simply exfoliated. There were large numbers of incompletely cornified cells immediately above the epithelial surface, and small crypts produced by the invading cancer tissue were also filled with many such exfoliating cells. Typical lamellar material of keratosis or parakeratosis was absent.

The cells had a high mitotic activity. They were large with eosinophilic cytoplasm (Fig. 92). Few keratohyaline granules were observed. There was a complete loss of cellular polarity to the basement membrane. The basal cells were of various sizes and were dispersed in several layers. In areas of acanthotic expansive growths of the cancerous tissue or in areas of active invasion of the underlying stroma, the cancer tissue was composed mostly of smaller cells with dark cytoplasm reminiscent of the metaplastic basal cells.

In a few instances the islands of invading carcinoma cells deep

in the stroma and in the myometrium had cystic spaces which were generally covered with only two layers of carcinoma cells. Some cysts contained mucoid material in addition to exfoliated cancer cells and cellular debris. These characteristics of the lesions suggested adenocarcinoma; however, the resemblance of the outer zone of the lining cells to the basal cells, the cytoplasmic basophilia, and the tendency for the cells to form sheets indicated squamous cell carcinoma. Inflammation, adhesions, and obstruction led to extreme defacement of the histological appearance of the uterine glands and the associated structures. A few normal glands thus were displaced and distorted, giving a false impression that they were invasive, despite the normal appearance of the individual cells. Such lesions were exceedingly difficult to diagnose when dysplastic cellular changes were superimposed.

The remainder of the glandular epithelium, when recognizable, and portions of the superficial epithelium which had not undergone squamous metaplasia or malignant transformation often had severe nuclear and cytoplasmic changes compatible with an advanced dysplasia of the columnar epithelium. Similar cellular changes were sometimes observed in the course of chemical induction of carcinoma as well as severe dysplasia in the endometrium of the rabbit. However, no evidence of invasion was observed in either instance.

Adenocarcinomas were very rare (Figs. 91 and 93).

In rare cases squamous cell carcinoma in situ was present in the uterine cervix in which invasive squamous cell carcinoma of endometrial origin had also developed. These observations suggest a possible difference in tissue susceptibility to the carcinogenic effects between

two different loci of the uterus, namely, the endometrium and the endocervix. The early experiments, in which a large number of leiomyosarcomas were induced, indicate similarly that smooth muscle cells were more vulnerable than the endometrial epithelium to the action of chemical carcinogens, once direct contact with such agents was permitted.

In the electron microscope, much cellular debris and many exfoliating cancer cells were present in the spaces which appeared in sections studied by light microscopy as portions of the endometrial cavity or cysts within tumor nodules. Such debris was composed of aggregates of keratin fibrils that were partly surrounded by membranes. No cytoplasmic organelles were recognized.

The exfoliated cells appeared swollen. They contained typical cytoplasmic organelles. In some places the cytoplasmic membranes were disrupted and the cytoplasmic contents protruded from these areas.

The cells constituting the luminal layer of the cancerous epithelium had undamaged cytoplasmic membranes. Often their cytoplasmic organelles were reduced in number. Mitochondria were often not clearly discernible. A few myelin-like figures were present. Rough-surfaced ergastoplasm was poorly developed and appeared in the form of a few electron-transparent dilated spaces surrounded by a single layer of membrane which was studded with a few ribosomal granules. A few multivesicular bodies were present immediately below the cell membrane. A small amount of granular material resembling keratohyaline granules was present in the central portion of the cells.

Most of the cells in the intermediate and basal zones of the

epithelium had only slightly indented cell membranes (Fig. 94). The desmosomes differed in number, size, and distance between one another. The cytoplasm contained a moderate amount of keratin fibrils which were gathered in bundles. The arrangement of these bundles was similar to that in the basal cells present in the metaplastic stage. Many ribosomes and polysomes were dispersed throughout the entire cytoplasm. The Golgi apparatus was small. A small amount of faintly electron-dense material was present within the cisternae of the Golgi apparatus. The rough-surfaced ergastoplasm was moderately developed. Some of its cisternae contained electron-dense material. In some cells a moderate dilatation of the cisternae was noticed. Many mitochondria were present. They contained faintly electron-dense matrix and typical cristae. Occasional small granules quite similar to ribosomes in size and electron density were present within the mitochondrial matrix. There were several membrane-bound electron-dense bodies containing myelin-like figures, granules, or many small vesicular structures.

The nucleocytoplasmic ratio was markedly increased. There was a moderate condensation of the chromatin material next to the inner nuclear membrane. The nucleoli were also enlarged. A hyperplastic appearance of nucleolonema was impressive. Many mitoses were present.

In most part, the basement membrane maintained its usual triple-zone formation (Fig. 95). The distance between two neighboring hemidesmosomes was about 200 μ and was more regular than that between the desmosomes connecting cells; however, in certain areas the outer dense stromal zone of the basement membrane could not be recognized, and correspondingly the basal cytoplasm of the carcinoma cells had many

irregular cytoplasmic processes projecting downward into the stroma. Hemidesmosomes or condensation of keratin fibrils on the cell membrane was not present in these processes (Fig. 96). Some of these processes appeared to be almost in direct contact with the stromal collagen fibers. These areas suggest a possible mechanism of carcinomatous invasion.

Tabulation of Data

The results are summarized in Tables 3, 7 and 12 according to the animal species. More details concerning the experiments are explained in the following 13 tables. In Tables 3, 7 and 12, all animals with normal, atrophic, hypertrophic or hyperplastic endometrial epithelium are listed under "Negative." The animals listed under "Inflammation" suffered from severe pyometra, in which the uterine mucosa was destroyed almost beyond histological diagnosis. "Total malignant lesions" include all carcinomas, carcinosarcomas, and sarcomas, but not carcinoma in situ. The percentage of malignant lesions produced was calculated from the number of animals examined in each group.

In Table 4, of 117 rabbits of the Dutch breed, three developed atypical clusters of glands, and in two others the pathological changes were sufficiently advanced to allow a diagnosis of carcinoma in situ. The eight carcinomas that developed were adenocarcinoma, and one had metastases to the lung. One leiomyosarcoma also had pulmonary metastasis. Most of the carcinomas developed after $1\frac{1}{2}$ years of observation.

The rabbits that received diethylstilbestrol had hypertrophic epithelium and stroma of the endometrium. There was a high incidence

pyometra in this group, due to Pasturella. The incidence of adenocarcinoma seemed decreased by stilbestrol administration.

Rabbits treated with 3-methylcholanthrene-impregnated strings developed dysplasia at seven months and carcinoma at eight months (Table 5). The tumor incidence was only slightly higher than that of spontaneous carcinoma (8/69 or 11.5%).

As noted from Table 6, the incidence of carcinoma was enhanced by the addition of diethylstilbestrol. Six well-differentiated and one undifferentiated adenocarcinoma were obtained. Two animals had distant metastasis. Dysplasia was present after nine months and most of the carcinomas were seen after ten months.

In the control group receiving injections of ethyl oleate alone, the occurrence of carcinoma was not affected.

Since among the normal control rats after 24 months only one rat had dysplastic tissue, a separate table was not made.

Many rats under the estrogenic treatment had marked hydrometra, which tended to become secondarily infected. After prolonged administration of diethylstilbestrol, some animals developed squamous metaplasia. A few had more advanced lesions.

As judged from Tables 8 and 9, estrogen apparently had no promotional or inhibitory effect on the development of the carcinogen-induced carcinomas of the rat endometrium. Table 10 suggests that diethylstilbestrol will retard the progression of carcinogenesis although it may not alter the ultimate yield of carcinomas. The high incidence of squamous cell carcinomas induced by the mixture of paraffin and polyvinyl chloride was totally unexpected. In view of

the data implicating plastic polymers with carcinogenic properties, polyvinyl resin may be considered to be the carcinogenic agent in these instances, not the paraffin (Table 11). There is an indication that the carcinomas produced with polyvinyl chloride may develop more slowly than those induced by the chemical carcinogen. The rate of incidence of malignant lesions beyond the tumor age, i.e., the date of the first noted appearance of the carcinoma including in-situ lesions, was 46.6% (14/30) in the methylcholanthrene group, 45.5% (16/36) in the methylcholanthrene-diethylstilbestrol group, and 45.5% (5/11) in the polyvinyl chloride group. There is little difference among the three groups. The one adenocarcinoma in the control group given ethyl oleate injections (Table 12) must be considered of spontaneous development.

A moderate number of leiomyosarcomas (Fig. 97) was noted in old mice of the control groups which were kept under observation for 18 months (Table 13). The rate of tumor incidence beyond the tumor age was 37.7% (5/13) in the normal control group and 40.0% (4/10) in the string control animals. The polyvinyl chloride-paraffin mixture had no carcinogenic effect. The leiomyosarcomas that developed produced a widespread peritoneal dissemination and a large amount of ascites fluid (5-8 ml.). The tumor was transplanted by intraperitoneal injections of the ascites fluid in young B6D2F₁ female mice. The tumor is now at the fourth generation. To attain a full yield of the ascites, it is necessary to wait 2-3 months after the transplantation. Although the growth is slow, transplantation is nearly 100% successful.

In the earliest experiments, in which the methods of Bonser and

Robson were followed, many leiomyosarcomas were obtained (see Tables 14 and 15). These approaches were therefore discontinued. The risk of traumatizing the uterus while injecting the oil-suspended methylcholanthrene was great. The sutures in the uterine horn led to retention of fluid and subsequent rupture of the uterus. This trauma exposed the smooth muscle cells in the myometrium directly to the carcinogen. In the second experiment, in which intact animals were used and the uterus was not tied, many squamous cell carcinomas were obtained (Tables 16 and 17). The simultaneous administration of diethylstilbestrol decidedly increased both the velocity of growth and the final yield of carcinomas. Such trends were also obvious in the short-term experiments as shown in Table 18.

The mice treated with diethylstilbestrol developed extreme hydro-metra but rarely developed an infection. After 573 days of treatment, marked distention of the urinary bladder, up to 3 cm. in diameter, was noted.

Spontaneous Endometrial Carcinoma in Rabbits

Senile atrophy.--Senile atrophy was noticed both in the epithelium and in the stroma (Fig. 98). The stromal atrophy was made evident by the reduction of the connective tissue cells, an increase in hyalinization, and fibrosis of the stroma. The epithelial changes included atrophy of the superficial epithelium, indicated by lack of secretion and cilia, and a decreased cell height. There was also a marked reduction in both number and size of the glandular lumens, and occasional glands showed cystic dilatation. Such glands were irregularly

Table 3.--Results on rabbits

	Total	Examined	Alive	* Negative	Inflammation	Metaplasia	Dysplasia	Carcinoma in situ	Adenocarcinoma	Sarcoma	Total malignant lesions (%)	
Normal control	117	69	32	51	3	1	3	2	8	1	9	13.0
Castrate	3	3		3								
Diethylstilbestrol	78	74		49	21	1		1	2		2	2.7
Methylcholanthrene	21	20		11		2	4		3		3	15.0
Methylcholanthrene + diethylstilbestrol	24	24		4	4	2	6	1	7		7	29.1
String control	10	9		8			1					
Injection control	14	12		11					1		1	8.3
Total	267	211	32	137	28	6	14	4	21	1	22	10.4

* Negative: The normal, atrophic and hyperplastic endometrium are all included.

Table 4.--Rabbits under observation (normal controls)

Observation (Months)	Normal	Inflam- ation	Atrophy	Hyper- plasia	Meta- plasia	Atypia	Carcinoma in situ	Carcinoma	Sarcoma	Total
1- 2	6		2							8
3- 4	1									1
5- 6	3		2		1					6
7- 8	1	2	3			1		1		8
9-10	4	1								5
11-12	1		1	2						4
13-14			3	1				1		5
15-16	2		4				1			7
17-18	1			1				2		4
19-20	2			1		1		1		5
21-22	4					1	1			6
23-24				1				2		3
25-26	1		1						1	3
27-28			2							2
29-30	1							1		2
Total	27	3	18	6	1	3	2	6	1	69

Table 5.--Rabbits treated with 3-methylcholanthrene

Months	Negative	Metaplasia	Dysplasia	Carcinoma	Total
4		1			1
5					
6					
7			1		1
8	1			1	2
9	2	1	2		5
10	2				2
11	2				2
12				1	1
13	2		1		3
14	1			1	2
15					
16					
17	1				1
18					
Total (20)	11	2	4	3	20

Table 6.--Rabbits treated with 3-methylcholanthrene and diethylstilbestrol

Months	Negative	Inflam- mation	Meta- plasia	Dysplasia	Carcinoma in situ	Carcinoma	Total
3	1	1					2
4	1						1
5	1						1
6							
7							
8		1				1	2
9		1		2			3
10	1			1		1	3
11				2		1	3
12			1		1	2	4
13						1	1
14				1			1
15			1				1
16							
17							
18							
19		1				1	2
Total	4	4	2	6	1	7	24

Table 7.--Results on rats

	Total	Examined	Negative	Inflammation	Metaplasia	Dysplasia	Carcinoma in situ	Squamous c. carc.	Adenocarcinoma	Carcinosarcoma	Sarcoma	Total malignant lesions (%)
Normal control	16	15	14			1						
Castrate	5	5	5									
Diethylstilbestrol	40	36	18	8	6	2	1	1				1 2.7
Methylcholanthrene	40	35	8	1	10	2	1	12		1		13 37.1
Methylcholanthrene + Diethylstilbestrol	41	40	1	6	12	5	2	13			1	14 35.0
Methylcholanthrene (castrate)	10	10		1	3	5	1					
Methylcholanthrene + stilbestrol (castrate)	10	9	1		2	4		2				2 22.2
Dimethylbenzanthracene (castrate)	5	4				2	1	1				1 25.0
Dimethylbenzanthracene + stilbestrol (castrate)	5	4		1	1	2						
String controls	16	12	1	4		2		5				5 41.6
Injection control	17	11	4	4	1		1		1			1 9.0
Total	205	181	52	25	35	25	7	34	1	1	1	37 20.5

Table 8.--Rats treated with 3-methylcholanthrene

Days	Negative	Inflam- mation	Meta- plasia	Dysplasia	Carcinoma in situ	Squamous cell carcinoma	Carcino- sarcoma	Total
61- 90			2	1				3
91-120								
121-150			2					2
151-180			1			1		2
181-210								
211-240	1		1				1	3
241-270	1		1	1	1	1		5
271-300	4					1		5
301-330						4		4
331-360			1			5		6
361-390								
391-420	2	1	2					5
Total	8	1	10	2	1	12	1	35

Table 9.--Rats treated with 3-methylcholanthrene and diethylstilbestrol

Days	Negative	Inflam- mation	Meta- plasia	Dysplasia	Carcinoma in situ	Squamous cell carcinoma	Sarcoma	Total
31- 60			1	1				2
61- 90				1				1
91-120								
121-150			1					1
151-180								
181-210	1		1			2		4
211-240				1		1	1	3
241-270		3	4	1	1	1		10
271-300		1	1	1		4		7
301-330		1				3		4
331-360		1	3		1	1		6
361-390			1					1
391-420						1		1
Total	1	6	12	5	2	13	1	40

Table 10.--Castrated rats with carcinogens (short experiments)

Months	Negative	Inflam- mation	Meta- plasia	Dys- plasia	Carcinoma in situ	Squamous cell carcinoma	Total
With diethylstilbestrol							
1	1		2				3
2				3			3
3			1	2			3
4		1		1			2
5							
6							
7						2	2
Total	1	1	3	6		2	13
Without diethylstilbestrol							
1		1	1	1			3
2			2	1			3
3				2	1		3
4				1	1	1	3
5				2			2
6							
7							
Total		1	3	7	2	1	14

Table 11.--Rats treated with a mixture of paraffin and polyvinyl resin

Days	Negative	Inflam- mation	Dysplasia	Squamous cell carcinoma	Total
121-180		1			1
181-240					
241-300					
301-360		1	2	3	6
361-420		1			1
421-480		1			1
481-540	1			2	3
Total	1	4	2	5	12

Table 12.--Results on mice

	Total	Examined	Negative	Inflammation	Metaplasia	Dysplasia	Carcinoma in situ	Squamous cell carcinoma	Adenocarcinoma	Adenoacanthoma	Carcinosarcoma	Sarcoma	Total malignant lesions (%)
Normal control	24	20	15									5	5 25.0
Castrate	13	10	10										
Diethylstilbestrol	47	37	36									1	1 2.7
Methylcholanthrene in oil (castrate)	50	35	8	10				1	2			14	17 48.6
Methylcholanthrene (uterus tied-castrate)	64	51	10	10	1	13	2	5	1			9	15 29.5
Methylcholanthrene	60	53	3	8	8	5	4	22	2			1	25 47.2
Methylcholanthrene+ diethylstilbestrol	40	36			2	2	1	24	2	4	1		31 86.0
Methylcholanthrene (castrate)	20	12		4	5	3							
Methylcholanthrene+ stilbestrol (castrate)	20	16			3	7	3	3					3 18.7
Dimethylbenzanthracene (castrate)	10	8			4	3		1					1 12.5
Dimethylbenzanthracene+ stilbestrol (castrate)	10	8			3	3		1	1				2 25.0
String control	20	14	5	5								4	4 28.5
Injection control	15	11	11									1	1 9.1
Total	393	311	97	37	26	36	10	57	8	4	1	35	105 33.7

Table 13.--Control mice (normal control, injection control and string control groups)

Days	Normal controls		Injection controls		String controls			Total
	Negative	Sarcoma	Negative	Sarcoma	Negative	Inflam- mation	Sarcoma	
61-120	4		1					5
121-180								
181-240	1		1			2		4
241-300								
301-360					1	1		2
361-420			4					4
421-480	2							2
481-540	2	1						3
541-600		2					1	3
601-660	3	1	4	1	4	2	3	18
661-720	1							1
721-780	1	1						2
781-840	1							1
Total	15	5	10	1	5	5	4	45

Table 14.--Castrated mice treated with 3-methylcholanthrene in oil suspension

Days	Negative	Inflam- mation	Squamous cell carcinoma	Adeno- carcinoma	Sarcoma	Total
91-120		5			3	8
121-150	2				2	4
151-180	2	1			5	8
181-210	1	1	1		1	4
211-240	1	2		1	3	7
241-270	1					1
271-300						
301-330	1					1
331-360						
361-390						
391-420		1		1		2
Total	8	10	1	2	14	35

Table 15.--Castrated mice treated with 3-methylcholanthrene (uterus tied)

Days	Negative	Inflam- mation	Meta- plasia	Dys- plasia	Carcinoma in situ	Squamous cell carcinoma	Adeno- carcinoma	Sarcoma	Total
1- 30		2		1					3
31- 60	1	5		1					7
61- 90	2	2		3	2				9
91-120	2			3		2		1	8
121-150	4		1	2		2	1		10
151-180		1		2		1		0	12
181-450									
451-480	1			1					2
Total	10	10	1	13	2	5	1	9	51

Table 16.--Mice treated with 3-methylcholanthrene

Days	Negative	Inflam- mation	Meta- plasia	Dys- plasia	Carcinoma in situ	Squamous cell carcinoma	Adeno- carcinoma	Sarcoma	Total
31- 60		3	2		1				6
61- 90		1		3					4
91-120						3		1	4
121-150		1	1		1	3			6
151-180		1				3			4
181-210		2				4			6
211-240					1	3	1		5
241-270						1			1
271-300						2	1		3
301-330			1			2			3
331-360									
361-390			1		1				2
391-420	2			1		1			4
421-450	1		1						2
451-480			1						1
481-510			1	1					2
Total	3	8	8	5	4	22	2	1	53

Table 17.--Mice treated with 3-methylcholanthrene and diethylstilbestrol

Days	Meta- plasia	Dysplasia	Carcinoma in situ	Squamous cell carcinoma	Adeno- carcinoma	Adeno- acanthoma	Carcino- sarcoma	Total
31- 60		1	1					2
61- 90		1		1				2
91-120				6		1		7
121-150				6				6
151-180				8		1	1	10
181-210	1			3	2	2		8
211-240								
241-270								
271-300								
301-330	1							1
Total	2	2	1	24	2	4	1	36

Table 18.--Castrated mice with carcinogens (short experiments)

Weeks	Inflam- mation	Meta- plasia	Dys- plasia	Carcinoma in situ	Squamous cell carcinoma	Adeno- carcinoma	Total
With diethylstilbestrol							
2		3					3
4		2	1				3
6			4				4
8			2	1			3
10		1	1	1			3
12				1	2		3
14			2		1		3
16						1	1
18					1		1
Total		6	10	3	4	1	24
Without diethylstilbestrol							
2	1	2					3
4	1	1	1				3
6		3					3
8	1	1	1				3
10	1	1	1				3
12		1	1				2
14			1		1		2
16			1				1
Total	4	9	6		1		20

distributed in certain areas, whereas in other portions of the endometrium no glands were seen.

In the electron microscope the atrophic changes were much more pronounced in the deepest portions of the glands (Fig. 99).

Most of the deep glandular cells had only a minimal amount of secretory vesicles. The cells were short were no cystic dilatations were present. The luminal cell surface was often convex even in the absence of secretory products. The number of microvilli was markedly reduced. The lateral cell membrane seemed straight, and the desmosomes were small; however, there was no cellular dissociation. The basal cell membrane was occasionally tortuous.

In the cytoplasm, the rough-surfaced ergastoplasm showed rather short profiles, and the lumens were frequently distended. Ribosomes were often abundant in both membrane-bound and free forms. In some cells, because of the paucity of other cytoplasmic organelles, considerable areas of the cytoplasm were occupied only by free ribosomes. Lysosomes were infrequently seen. The Golgi regions were rather small and were seen in the apical cytoplasm. A few multivesicular bodies were present in the subluminal zones. The mitochondria showed a normal parallel pattern of cristae, a slightly electron-dense matrix, and some small intramitochondrial bodies (Fig. 100). The cytoplasmic fibrils appeared fairly abundant. Small lipid droplets were rarely seen. No glycogen granules were noted.

The nucleocytoplasmic ratio was increased. The nuclear membrane was smooth, with only few invaginations. The karyoplasm was rather diffusely distributed. The nucleoli were not enlarged.

The cellular morphology was somewhat different in the cystic linings (Fig. 101). Naturally the cells were flat. Cilia were less frequent. Microvilli were occasionally visible and were probably slightly more numerous than in the deep glandular portion. The luminal cell surface was flat or, rarely, convex. In contrast to those of the deep glandular cells, the lateral cell membranes were very tortuous and were bound by very prominent desmosomes. Often widening of the intercellular space was present. The nuclei showed occasional inclusions in the form of round bodies consisting of medium electron-dense granules, and also occasional indentations of the nuclear membranes. The nucleoli rarely showed prominent nucleolonema. The cytoplasm showed a marked condensation of its ground substance in a zone up to 300 μ in thickness. The underlying portion of the apical cytoplasm showed a moderate number of small vesicles and some multi-vesicular bodies. Both the rough-surfaced ergastoplasm and the Golgi apparatus were better developed than in the deep glandular cells. The cytoplasm was often dense, mainly due to an increased amount of cytoplasmic fibrils. Free ribosomes appeared abundant.

Cystic hyperplasia.--Cystic hyperplasia of the rabbit endometrium could be recognized by its gross appearance since the endometrial surface was covered with multiple small cysts containing clear fluid.

In light microscopy, the endometrium showed numerous cysts covered by ciliated and non-ciliated cells (Fig. 102). These cysts were considered as derived from the upper portions of glands, since in the deeper portion of the endometrium there were a few small glands composed of cells with pale-staining cytoplasm and without cilia. Both

the surface and the lining epithelium of the cysts were flat, due to pressure from the contents of the cysts, and showed moderate eosinophilia of the cytoplasm. No cellular or structural abnormality of the glands, whether cystically dilated or not, was found.

In the electron microscope, because of the many distended cysts, the lining epithelial cells appeared moderately flattened. The superficial epithelial cells which covered such cysts were also flattened.

The apical or luminal cell surface of the superficial cells was round. Many cilia were seen or secretory function was moderately active (Figs. 103 and 104). Microvilli were fairly long in the superficial epithelium. The lateral cell membrane of the superficial cells was moderately tortuous, but the intercellular spaces were narrow. Desmosomes were fairly prominent in the apical portion but not elsewhere. The apical cytoplasm was slightly decreased in height. To the slightly swollen rough-surfaced ergastoplasm many granules were bound. The Golgi apparatus was very well developed, and small vesicles were seen throughout the cytoplasm. In the apical portion, the appearance of the cisternae of the Golgi complex suggested that they were being transformed into single-membrane-surrounded secretory vesicles with electron-pale material. Phagosomes were rare, as were multivesicular bodies. Lysosomes were very seldom seen. The mitochondria were slender, with uniform crista formation and dense matrix. Intramitochondrial bodies were prominent. The mitochondria in the ciliated cells were longer. The cytoplasm seemed fairly abundant in fibrils. The apical portion of non-ciliated cells showed a marked increase in secretory vesicles, which were single-membrane-surrounded and contained what appeared as

fibrillary material after fixation and probably represented mucus. The middle portion of the cytoplasm also contained many organelles. The basal cytoplasm was not rich in Golgi apparatus, but many mitochondria and rough ergastoplasm were seen. In addition, there were many single-membrane-surrounded bodies of medium electron density often associated with granules, particularly at their membranes. Since these were seen only in the secretory cells and corresponded to the PAS-positive material in the light microscopy slides, they may represent mucoprotein, probably a storage form.

The nuclei had fairly smooth outlines. The karyoplasm was fairly diffusely distributed, and occasional ringlike membranous structures containing some small granules were seen in the karyoplasm. The diameter of these tiny granules was about 20 μ . Condensation of karyosomes was not particularly prominent. Nucleoli showed a lesser amount of pars amorpha. The nucleocytoplasmic ratio was estimated at around 1:1.

The glandular portion of the epithelium which was undergoing cystic change was almost identical with the surface epithelium, which was so much compressed by the underlying cysts in its structure. The cell size was almost identical, both in height and width. The luminal cell surface had as many cilia, and the cells contained as much secretion as the cells of the superficial epithelium. However, microvilli seemed fewer and shorter in the glandular portion. The cell surface tended to become flat rather than convex. The lateral and basal cell membranes and their associated structures, such as desmosomes, were also similar to those of the surface cells. In the cytoplasm, the

rough-surfaced ergastoplasm seemed to have narrow profiles, and ribosomes were abundant. The Golgi apparatus, dense bodies, and secretory vesicles were all similar to those of the surface cells. The nuclei did not differ, but because of the reduction of the apical cytoplasm the nucleocytoplasmic ratio was slightly reduced.

Papillary and glandular hyperplasia.--Papillary hyperplasia was characterized by a finely granular or velvety gross appearance of the endometrium. The histological picture of this change was an overgrowth of the superficial epithelium with many papillary proliferations, so that most of the papillary projections were covered by superficial cells, and many cilia were seen. The glandular components lined by the cells with paler cytoplasm were rather few. Often moderate secretion was found. This stage of papillary hyperplasia seemed to persist for years without any transformation towards malignancy. This stage may not represent reactive hyperplasia since there was no evidence of hyperestrinism in the morphology of the endometrial stroma and the ovaries were not particularly hyperactive, containing an average number of small follicles. No such papillary hyperplasia was associated with carcinomatous endometrium.

Glandular hyperplasia involved the surface and the glandular epithelium, but there were no papillary projections (Fig. 105). The ratio between epithelium and stroma was increased, and the glandular spaces were very closely arranged. The glandular cells were tall and uniform in appearance. Many ciliated cells were seen. No atypia was found.

In the electron microscope the epithelial cells showed many cilia

and moderate secretory activity. Their ultrastructure bore no morphological resemblance to carcinoma cells. The glands were closely arranged and were lined by tall cells (Fig. 106). No diversity of the cellular appearance was seen. The dilatation of the glands and the amount of secretion of the cystic hyperplasia were lacking. The non-ciliated cells showed a mild secretory activity. The luminal, lateral, and basal cell membranes showed no particular deviations from the normal cells, although the lateral cell membranes tended to show less remarkable tortuosity. In the apical cytoplasm, there were many electron-pale secretory vesicles surrounded by a single membrane (Fig. 107). The membranes were often disrupted, and the contents appeared very finely granular to fibrillary. These vesicles were limited to the very subluminal areas and were much fewer than in cystic hyperplasia. Denser membrane-surrounded bodies were seen in the basal cytoplasm of the non-ciliated estrogen-stimulated cells, but they were much fewer than in cystic hyperplasia. Both rough-surfaced ergastoplasm and Golgi apparatus were well-developed. The profiles of the rough-surfaced ergastoplasm seemed narrow, but there were abundant granules and there were also abundant free ribosomes.

In the apical cytoplasm, phagosomes were less frequent, but the lysosome-like bodies were more frequent than in normal epithelium. The mitochondria were numerous, with regularly arranged cristae, dense matrix, and small intramitochondrial bodies. A moderate amount of cytoplasmic fibrils and occasional lipid droplets were seen in the basal cytoplasm.

The nuclei seemed somewhat enlarged but not otherwise unusual,

and the nucleocytoplasmic ratio was increased in some cells. The karyoplasm showed no condensation, but nucleoli were more prominent, particularly in the nucleolonema. No particular increase was noted in the amount of pars amorpha.

Adenocarcinoma in situ.--Adenocarcinoma in situ was observed in two old animals.

Both dysplasia and carcinoma in situ were noted in the deep portions of glands, usually associated with moderate or severe atrophy of the endometrium. The glands showed some cystic dilatation. The atypical glands were very irregularly distributed and were seen in closely packed clumps (Fig. 108). The contours of such glands were also very irregular since the height and width of the lining cells of the glands varied greatly. These cells had relatively large nuclei, and the cytoplasm was eosinophilic. Neither cilia nor secretions were seen in such glands. Many exfoliated epithelial cells and some chronic inflammatory cells were seen within the glandular lumens (Fig. 109). Mitoses were not prominent in the glands of either dysplasia or carcinoma in situ.

The difference between dysplasia and carcinoma in situ was more or less quantitative rather than qualitative, i.e. many more glands were involved when the endometrium showed in-situ carcinoma.

In the electron microscope, the cells showed a moderate range in height and width. If the glandular spaces were dilated, the cells were flat. In areas of the same lesion where glands were less distended, the cells assumed either columnar or cuboidal shape (Fig. 110).

The luminal cell surface was in general flat, with no or very few

microvilli, where were short and plump. Some cells had undulating superficial cell surfaces. No cilia or secretory activity were present. The lumens of such glands contained many exfoliated epithelial cells undergoing degeneration and some inflammatory cells. The lateral cell surface was tortuous in some places and straight in others, and widenings of the intercellular spaces were often noted. Occasionally the cellular dissociation was so marked as to produce wide intercellular spaces lined by many microvilli. The basal cell membranes were straight in most areas. In some areas very irregular indentations of the basal cell membranes could be seen, with saw-like notches and an accompanying condensation of the dense outer stromal zone of the basement membrane. No defect, however, was found in the basement membrane.

The cytoplasm of the epithelial cells was often even more electron-dense than the nuclei, due partly to the abundance of cytoplasmic fibrils. The rough-surfaced ergastoplasm was often seen with widened lumens, and its profiles looked shortened. The granulation was adequate and free ribosomes were abundant. The Golgi apparatus was fairly well-developed, and numerous small, smooth vesicles were seen throughout the cytoplasm. The mitochondria were numerous, slender, and retained their usual structure. Most of them showed the regular arrangement of cristae and dense matrix. The intramitochondrial bodies were small. In rare mitochondria the cristae ran parallel to the longer axis. Infrequently swelling of mitochondria was seen. Enlargement of the intramitochondrial bodies was seldom seen. In this stage a marked increase in size and number of lysosome-like bodies was

noted (Fig. 111). Most of these bodies were surrounded by a single membrane and contained dense material with or without inner granular, membranous or tubular structures. Such an abundance of lysosomes or similar structures has been encountered only in the in-situ stage of spontaneous endometrial carcinoma. Occasionally very small lipid droplets were seen. No glycogen granules were present.

The nuclei in this stage showed moderate diversity. There was an increase in the infolding of the nuclear membranes, and the karyoplasm was often dense. The nucleoli became denser, particularly in the pars amorpha, and the nucleolonema was also often prominent. Circular membrane-like intranuclear inclusions were seen in some cells.

Adenocarcinoma.--Spontaneous adenocarcinomas were observed in eight animals. The adenocarcinoma was often polypoid and multifocal. In some instances moderate ulcerations were seen in the center of carcinomatous polyps (Fig. 113). The uterus was usually nodular (Fig. 112) and sometimes showed retention of fluid due to obstruction of the lumen by the tumor nodules. Many tumors invaded the underlying myometrium and often broke through the serosa. Serosal implantation on the mesometrium was noted in several cases; in rare cases tumor implantation was found on the vaginal mucosa. Distant metastases were rare and were usually in the form of peritoneal disseminations. Two animals had pulmonary metastases. No lymph node or liver metastasis was noted.

The histological pattern was always that of a well-differentiated adenocarcinoma. A single carcinomatous nodule consisted of very closely arranged small glands. Each of these glands showed interweaving cords

of highly eosinophilic small cuboidal cells. Sometimes these cells were arranged in trabeculae, sometimes in papillary projections. Frequently tiny buddings of the glands into the surrounding stroma could be seen. The stroma encircling these carcinomatous nodules was of loose connective tissue without desmoplasia, although some hyalinization took place.

The malignant cells showed a moderately dark-stained cytoplasm, a large vesicular nucleus with prominent nucleoli, and a markedly increased nucleocytoplasmic ratio (Fig. 115). Numerous mitoses were seen. In general, the carcinoma cells showed no remarkable pleomorphism and were rather uniform in size and appearance.

The surface epithelium overlying the carcinomatous nodules was free of malignant transformation but was markedly to moderately atrophic, probably due to pressure by the growing tumor (Fig. 114). The remainder of the endometrium was moderately atrophic. The carcinomas were never associated with papillary, glandular, or cystic epithelial hyperplasia.

In the electron microscope, the significant malignant changes were: (1) increase in nucleocytoplasmic ratio; (2) prominence of the nucleolus; (3) marked simplification of cytoplasmic structures; (4) loss of highly specialized cellular function and functional units such as secretion and motility or secretion granules and cilia (Fig. 116).

Because of marked dedifferentiation and loss of organization of cells showing malignant change, it was very difficult to recognize the cancer cells solely from the submicroscopic pictures. They could be recognized by low-power and thick-section examinations, in which the

formation of cell clumps, characteristic of the cells of epithelial origin, could be seen. Often the open spaces seen among the cell clumps simulated glandular lumens. While identification of individual cancer cells by light microscopy was not impossible, it was often difficult even in the thick sections.

Carcinoma cells were cuboidal and varied in size. In some places they formed simple cuboidal epithelium, in other areas, stratified cuboidal epithelium. In many areas the basement membranes were so tortuous as to give an extremely complex appearance to the glands. The cells were often very much dissociated, and there were large intercellular spaces frequently indistinguishable from the luminal spaces. However, the cell population was rather uniform, except for those degenerating at the luminal surface, and no distinct differentiation between the A and B cells of Caspersson could be made.

The luminal cell surface protruded only slightly and possessed very scanty and short microvilli. Occasionally many microvilli were seen on the luminal cell surface where many cells had already reached hypermaturation and had started to degenerate as shown by cytoplasmic condensation possibly due to water loss. Some cells on the surface appeared autolyzed, having no luminal cell membranes, which had possibly been dissolved, and their cytoplasmic organelles were swollen and released into the lumen. This phenomenon, interpreted as hyperhydration, was the second type of degeneration of surface cells, the first being dehydration and cellular shrinkage.

The lateral cell membranes had only little tortuosity and were parallel except for approximately $4\ \mu$ of the basal portion, where the

cell surfaces were moderately winding and the intercellular spaces were widened. At the basal portion it measured up to 600 m μ . Just below the lumen, the intercellular space was often much widened and measured up to 1.2 μ . The desmosomes were limited to the apical portions. They were less fibrillar and stained very faintly. The basal membrane in most parts was formed by the usual three layers. Often there were irregular pseudopod-like processes of cytoplasm growing toward the mesenchyme, and the basement membrane curled remarkably (Fig. 118). At this point the transparent middle portion of the membrane disappeared and the basal cytoplasmic membrane was in direct contact with the dense outer layer, which often appeared markedly thickened and compressed. There were also many definite defects of the middle and outer zones of the basement membrane, indicating sites of microinvasion where the cytoplasmic membrane directly invaded the mesenchymal spaces.

The structural components of the cytoplasm were greatly decreased and differences in distribution between the apical and basal portions of the cytoplasm were less marked. Mitochondria, Golgi apparatus, rough- and smooth-surfaced ergastoplasm and ribosomes were all present. Lysosomes were rare.

The subluminal zone of the cytoplasm was slightly electron-dense, measured approximately 100 m μ , and lacked fine structures. Only few small pinocytotic vesicles were encountered, indicating decreased pinocytotic activity. No cilia were present.

The Golgi apparatus was localized more in the upper portion of the cytoplasm. Over all, the number of Golgi apparatus was slightly decreased, as was the size of the individual organelle (Fig. 117).

Mitochondria were also more numerous in the apical portion of the cytoplasm, though the basal portion contained them in moderate numbers. They were relatively short and often round. The cristae were regularly transverse, mostly completely connecting both outer walls. The mitochondrial matrix was moderately electron-dense and very finely granular. Intramitochondrial microbodies were very rare. The smooth-surfaced endoplasmic reticulum was poorly developed. They were usually seen in the apical portion of the cytoplasm. The diameter ranged between 60 and 80 μ . The rough-surfaced ergastoplasm was also decreased. In many portions it was devoid of ribosomes. The free ribosomes were only slightly decreased in number and the size of each was little altered. Only rarely were multivesicular bodies and lysosomes seen.

The middle portion of the cytoplasm was very poorly equipped with organelles.

The basal portion of the cytoplasm differed little from the middle portion, although mitochondria were more numerous. Tonofibrils were not very prominent in any portion of the cytoplasm.

The nucleus was large compared with the amount of cytoplasm and usually had smooth membranes without much infolding. The nucleolus was very prominent and electron-dense. The nucleolonema was clearly seen in many nuclei, while on the other hand the enlargement of the pars amorpha was not very striking.

CHAPTER IV

DISCUSSION

Nuclear morphology has long been considered as one of the most prominent features of malignant cells. The enlargement of the nucleus, according to Oberling and Bernhard,¹ is the result of a complexity of factors, such as the type of tissue from which the tumor is originating, the extent of polyploidy of the chromosomes, and the rate of metabolism of the tumor cells. This increase in nuclear mass is not characteristic for cancer cells alone, since it is also observed in non-malignant cells as the result of injury (liver cells), hypertrophy (heart muscle), and radiation (fibroblasts). The meaning of the increased infolding of the nuclear membrane of malignant cells, which was so evident in our ultramicroscopic studies, is still obscure.

The well-known nucleolar enlargement of cancer cells is also present in non-neoplastic cells, as for instance in hepatic cells under toxic influence and during regeneration.^{2,3} The nucleolus consists of two ultrastructural components--the cord-like nucleolonema and the diffuse pars amorpha. The former is mainly composed of ribonucleoproteins gathered along strands of deoxyribonucleoproteins. The latter is disc-like and is mainly deoxyribonucleoprotein. Using tritium-labeled thymidine, Granboulan and Granboulan⁴ showed active DNA synthesis in the nucleolus, apparently in both components. In the dysplastic and malignant cells of our experiments both nucleolonema and pars amorpha were enlarged, suggesting an increased synthesis of both nucleic acids.

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Another ultramicroscopic change of the nucleus of malignant cells was the appearance and distribution of intranuclear bodies. A significant increase of the membranous bodies was observed in dysplastic and malignant cells. The nature of these bodies is not known. On the basis of his observation on ascites hepatoma, Hoshino⁵ suggested that these bodies represent the infolded inner nuclear envelope. Similar bodies were also present in the human endometrial carcinoma,⁶ experimental hepatoma,⁷ rabbit carcinoma,⁸ hamster melanoma,⁹ and several other human carcinomas.^{10,11} Different from these are the denser and smaller parachromatic bodies described by Leplus and others,¹² by Watson,¹³ and also observed in our experiments but to which we attached no specific significance.

After osmium fixation, the major portions of the nucleus consist of the homogeneously fine granular karyoplasm, which is condensed in some areas adjacent to the nuclear envelope (karyosomes). Under higher magnification deoxyribonucleoproteins can be demonstrated as fine, diffusely scattered fibrils 10 m μ in thickness. The use of aldehyde prefixation, the application of enzymatic digestion with different nucleases,^{9,14-16} and special staining procedures with certain heavy metals such as indium¹⁷ and bismuth¹⁸ may throw more light upon the ultrastructure of the nucleus and the basic changes of the cancer cell nucleus that have never been demonstrated in the electron microscopy.

The role of the nucleus during chemical carcinogenesis has been studied by various biochemical methods. Brookes and Lawley¹⁹ recently showed a relationship between the carcinogenicity of certain hydrocarbons and the concentration of these substances attached to

deoxyribonucleic acids. By using molecular models, Boyland and Green²⁰ postulated that molecules of 3,4-benzpyrene can be fitted on the helix of DNA. Earlier Heidelberger and Davenport²¹ demonstrated an interaction between 1,2,5,6-dibenzanthracene and DNA isolated from the mouse skin. These results allow much speculation as to the interaction of nucleic acids with carcinogenic hydrocarbons, but the nature of the chemical combination has not been demonstrated. The fact that tritium-labeled 3-methylcholanthrene did not localize this substance in the nuclei is contradictory to such biochemical concepts. By utilizing a new technique, such as freeze-drying,²² it might be possible that in vivo autoradiography can be brought into the dimension of electron microscopy.

A second trend in biochemical investigations has been directed towards a mode of action of the chemical carcinogens not involving a direct chemical bondage. The data obtained in recent years indicate that the carcinogenic hydrocarbons may induce enzyme activities and increase the incorporation of amino acids into ribosomes.²³⁻²⁷ The abolishment of these actions by addition of puromycin or actinomycin D suggests that the hydrocarbons act on the synthesis of DNA-dependent RNA and the synthesis of ribonucleoproteins. Swift¹⁶ described two types of ribonucleoproteins in *Drosophila* salivary chromosomes--a chromosome-associated particle and a nucleolar particle--that had an appearance similar to cytoplasmic ribosomal proteins. He inferred that the former contained messenger RNA and the latter ribosomal RNA. The morphological study of the DNA-dependent RNA and messenger RNA represents a challenge for future electron microscopy. With future

improvement of fixation and other techniques, such studies of the nucleoproteins in the nucleus of cancer cells may be of utmost importance.

The ultramicroscopic changes occurring in the cytoplasm during the process of carcinogenesis seem more prominent than those occurring in the nucleus and involve all organelles visualized in the electron microscope. The most obvious changes observed were in the mitochondria--the principal sites of metabolic enzyme activity of cells. These morphological changes may reflect changes in the enzymatic activities.²⁸ Swelling of the mitochondria with disarrangement of the cristae, a frequent observation in our experiments, resembled those observed in hepatic carcinogenesis^{29,30,31} and in human endometrial carcinoma.^{32,33} According to Oberling and Bernhard,¹ it is one of the most frequent findings in cancer cells and may be due to the fragility of these organelles, or it may represent the reversible state of swelling due to the deficiency of ADP or ATP.³⁴

Scarpelli and von Haam³⁵ and Clyman³⁶ observed osmiophilic intramitochondrial bodies in 3-methylcholanthrene-treated uterine cervical epithelium of mice in the precancerous and cancerous stages. Similar intramitochondrial bodies were noted in the chemically induced mouse skin carcinoma.^{38,39} This change is not specific for hydrocarbons since croton oil³⁹ and Tween 60⁴⁰ produce similar inclusions in certain strains of mice. According to Nakai et al.,³⁹ the bodies are transient; they appeared as early as two days after a single application of 7,12-dimethylbenzanthracene and disappeared two weeks later. Burt et al.⁴¹ observed similar bodies in the methylcholanthrene-treated

intestinal epithelium of mice during the precancerous stage. Pillai⁴² did not observe similar bodies during the epidermal carcinogenesis with methylcholanthrene in the newt. No large intramitochondrial bodies were present in cells of rabbits or rats, suggesting that a genetic factor may be involved in their formation.

Alterations in the ergastoplasm during carcinogenesis have been observed by many workers and may consist of the disappearance of the rough ergastoplasm and a temporary loss of free ribosomes.^{29-31,43-45} In our experiments the ergastoplasm appeared less developed and vesicular and the numbers of free ribosomes were decreased. The cytoplasm of the dysplastic cells and carcinoma in situ cells appeared dense due to condensation of cytoplasmic ground substance and increased tonofibrils.

Judging from the markedly swollen mitochondria and the empty and vesicular ergastoplasm, the dark cells seen in carcinoma were interpreted as degenerating cells undergoing dehydration and shrinkage (coagulative necrobiosis) rather than representing Caspersson's Type A cells, as discussed by Nilsson in the human endometrial carcinoma.^{32,33} After fixation with glutaric dialdehyde, the presence of two types of cells with different ground substance density is more obvious than after osmium fixation. Such differences may be associated with the preservation of cytoplasmic micromolecular structures. On the other hand, the extremely electron-pale cells may indicate overhydration of the cytoplasm or hyperhydration necrobiosis.

The Golgi apparatus had the least change, although some dysplastic

cells had an enlarged Golgi apparatus. Mikata and Luse³¹ observed a decrease in size during hepatic carcinogenesis.

The lysosomes were moderately increased in both endometrial dysplasia and carcinoma in rabbits and carcinoma in situ in mice. In the human endometrial carcinoma lysosomes are abundant,^{32,33,46,47} Pillai⁴² considered the abundance of lysosomes during epidermal carcinogenesis as characteristic of neoplastic cells. During hepatic carcinogenesis the appearance of lysosomes followed the stage of mitochondrial swelling and degeneration.⁴⁸

The process of invasion by carcinoma could be demonstrated in the basal portion of the cytoplasm either by the lack of an outer zone of the basement membrane or an abnormal condensation of this area. Using ferritin-bound antibodies to isolated rat cancer basement membrane, Pierce et al.⁴⁹ observed that the ferritin was located not only in the basement membrane of the cancer cells but also in the rough-surfaced ergastoplasm. This is one evidence that at least one component of the basement membrane is formed by epithelial cells. Studies by Hay and Revel⁵⁰ indicate that some components of the basement membrane are formed by fibroblasts. Such interaction of epithelial cells and the basement membrane is suggested in our electron micrograph where the electron-dense material appeared simultaneously within the cytoplasm and the outer zone of the basement membrane at the site of hemidesmosomes. In carcinoma the loss of organization of epithelial reticulin may be the cause of the defects in the normal triple layer of the basement membrane. The process of invasion may not necessarily be the result of destruction of the basement membrane by the malignant cell, but

a passive phenomenon secondary to failure of the cancer cells to participate in basal membrane construction.

Rabbits and mice developed histologically different carcinomas; however, they showed some common features: (1) the ultrastructural alterations of the cells were the most severe in dysplasia, and (2) estrogens enhanced the process of chemical carcinogenesis.

A marked reduction in number of the cytoplasmic organelles and the appearance of huge intramitochondrial bodies in mice and of defective mitochondria in rabbits characterized dysplasia. Such severe ultrastructural changes depended largely upon the severity of cellular injury. This is not interpreted as an expression of a neoplastic transmutation since the superficial cells of the rabbit endometrium had greater cellular disorganization than the glandular cells. The glandular cells retained an organized system of organelles yet subsequently became malignant. In the spontaneous trout hepatoma, the presence of lysosomes, indicating cell injury, was most prominent in the preneoplastic stage,⁴⁸ and in hepatic carcinogenesis hepatoma cells again showed glycogen deposits³¹ and ribosomes lost during the early phase of carcinogenesis. Peculiar mitochondrial changes in mice were limited to the dysplastic or preinvasive stage,^{35-39,41} and in the iron-dextran-complex induced sarcoma malignant fibroblasts were free of iron-containing lysosomes which had been prominent in the preceding stage.⁵¹

The carcinoma in situ cells and the invasive carcinoma cells in rats and mice were almost identical in their appearance. Both assumed a basal or parabasal cell type. Sandritter and his co-workers⁵²

observed by means of cytophotometry that the human cervical carcinoma cells and in-situ carcinoma cells were very similar in their DNA contents.

While the glandular epithelium of the rabbit developed a malignant and invasive transformation, the superficial cells did not become invasive. The reverse was true in mice and rats, which developed malignant lesions from the superficial cells. Thus the tissue susceptibility seemed definitely different in different locations, and the origin of carcinoma cells seems to determine the histopathological forms.

Regardless of the histological pictures, the common feature of the cancer cells was a simplified appearance of their cytoplasm without the functional units, such as cilia or secretory vesicles. Therefore Oberling and Bernhard¹ viewed this process of carcinogenesis as one of cytoplasmic dedifferentiation.

It can be said that the neoplastic cells seem to have recovered from the severe cellular damages inflicted by the carcinogenic agents during the preceding dysplastic stage. They have now reoriented themselves at a lower level of differentiation and regained the power of survival and proliferation but not their previous normal cellular behavior.

The ultrastructural diversity of dysplastic cells is in contrast to the uniformity of cancer cells. One might consider that the cancer cells belong to one clone while the dysplastic cells constitute different clones, and that the chemical carcinogenesis is actually a process of clonal selection. A similar idea was recently put forth by

Prehn,⁵³ whose theory depends upon the presumption that cellular carcinogen-combining factors are identical with cellular growth control factors. Cellular autonomy leading to uncontrolled progressive growth will appear in those cells with the least amount of growth control factors since they have been least injured by the chemical carcinogens. While these experiments are no proof of this hypothesis, it is important to note that the most active rabbit tumor cells originated in the glandular portions of the endometrium--an area with fewer dysplastic changes than the superficial cells. This observation could well support the hypothesis that the cancer cells developed from a cell clone that suffered much less toxic damage from the chemical carcinogens.

The nature of the peculiar mitochondrial change in mice has not been elucidated, although many reports have appeared on this phenomenon. The homogeneous electron density of these bodies suggests that they may possibly contain lipid. These bodies may represent a pathological accumulation of phospholipids in the matrix of the mitochondrion secondary to a failure in the incorporation of lipid components into the structure of the mitochondrial membranes. Since both protein synthesis and lipid synthesis occur within the mitochondria³⁴ and occur independently,⁵⁴ such an accumulation may further reflect deficiency of structural protein synthesis in the mitochondria. This might be another suggestion for the protein depletion as originally proposed by Miller and Miller.⁵⁵ This could be also explained by the uncontrolled overproduction of lipid by a possible inactivation of the repressor system, if such is present for phospholipid metabolism.

Recent investigations have demonstrated that mitochondria contain DNA and are self-replicating.^{34,56-59} This organelle certainly deserves more attention in carcinogenesis. It is possible that mitochondrial DNA may combine with the carcinogenic hydrocarbons, resulting in a mutation of this presumably cytoplasmic gene earlier than that of the nuclear gene. Assuming that these genes also undergo segregation and mutation,⁶⁰ this alone could lead to a significant degree of clonal selection in carcinogenesis. This would at least in part be in accordance with Hadow's theory.⁶¹ Morphological alterations of mitochondria are associated with the gene mutation of the organelle in yeast.^{62,63} The mitochondrial changes in dysplasia may represent some mutation in the mitochondrial gene.

As pointed out, the activation of chemical carcinogenesis in rabbits and mice by estrogens was very striking, particularly in mice. Since estrogens alone did not induce carcinomas, they are considered to be a promoting factor rather than an initiating factor or complete carcinogen. A highly hypothetical concept by Green⁶⁴ stated that the extreme hyperplasia of the cells under hormonal stimuli leads to nutritional deficiency of the cells, which in turn lose their specific antigens. Thus they are less well controlled by the immunological mechanism and tend to become autonomous in growth. In our study the cells under estrogen stimuli were hyperplastic and often extremely swollen. Secretion or cilia were present, and no loss of the structures attached to the cell membranes was obvious. In rat endometrium metaplastic tissue developed but this tissue did not become malignant. The data in this experiment does not favor the Green theory of carcinogenesis.

It is very interesting that in recent years biochemical studies have shown that both methylcholanthrene and estrogen act on the synthesis of DNA-dependent RNA and that of protein in ribosomes.⁶⁵ Such action can be abolished by the addition of actinomycin D and puromycin. It is therefore possible that they may work in an additive manner.

It is interesting that in the rat experiments only, estrogens produced no increase in the chemical induction of carcinoma, that polyvinyl resin produced carcinomas, and that the cells had the least alterations in their ultrastructure during the course of carcinogenesis, particularly in the dysplastic stage. Perhaps the induction mechanism of carcinogenesis by methylcholanthrene and by polymers differs. It is possible that polymers, as many past investigations have suggested,^{66,67} may produce some alterations on the cell membrane or the adjacent cytoplasm. Such alterations may produce altered metabolites in the cells, which in turn activate certain repressor systems, as proposed by Monod and his group⁶⁸ in a bacterial cell. Such repressor systems could act on genes (DNA) or operator messenger-RNA to change the cellular behavior. This would be in agreement with the idea of the protein deletion theory in carcinogenesis, as proposed by Miller and Miller. Thus it may not be necessary to postulate the direct combination between the carcinogenic hydrocarbons and the nuclear DNA to explain their carcinogenic property.

The ultrastructure of the normal and malignant human endometrium has been studied extensively.^{32,33,46,47,69-80} The rabbit endometrium and the human endometrium are composed of ciliated cells and non-ciliated cells and differ in several respects. The rabbit endometrial cells

lack glycogen deposits and have definite secretory vesicles. During secretion, only the vesicles are released; in human endometrium, some of the cytoplasm is also released from the cells. The rootlets of the cilia do not have cross-striations. There is no spontaneous luteal phase and no associated nucleolar inclusions^{80,81} or giant mitochondria.^{78,82}

The senile atrophy of the human endometrium was described by Sirtori and Morano in detail.⁴⁷ There was a general reduction in the number of cytoplasmic organelles. They noted two types of cells: one type of cells had deformed mitochondria and swollen ergastoplasm; the other type had a marked paucity of cytoplasmic organelles. Borrell et al.⁸⁴ examined an atrophic endometrium in an infertile patient and noted the presence of fewer organelles and shortened microvilli. In our study, the rabbit endometrium also had a decrease in the number of organelles although the individual structure retained their normal appearance. The second type of cells which was described by Sirtori and Morano in the human endometrium as necrobiotic cells was not observed.

The glandular cystic hyperplasia of the human endometrium was described by Wessel.⁸⁵ He noticed a similarity between the hyperplastic epithelial cells and the normal cells present during the late proliferative phase. In the rabbit endometrium, both glandular hyperplasia and cystic hyperplasia were noted. The cells in cystic hyperplasia had moderate secretory activity and those in glandular hyperplasia had less activity. In both conditions the cytoplasmic organelles were abundant and the cells appeared active. There was no irregularity of

the epithelial architecture or cellular abnormality. Therefore this lesion was not considered to be a premalignant lesion.

The previous studies on the human adenocarcinoma in situ of the endometrium by means of exfoliative cytology showed significant changes in nuclei.^{86,87} There were marked changes in the nucleocytoplasmic ratio and enlargement of the nucleoli. Nuclear hyperchromatism was not noted, but eosinophilic changes of the cytoplasm were often seen. These observations correspond, to a certain extent, to the current studies of rabbit tumors, in that there were changes in the nucleocytoplasmic ratio, and the cytoplasm showed rather more electron density than the nucleus.

The ultrastructure of adenocarcinoma of the human endometrium has been examined by several authors.^{32,33,36,37} Nilsson and co-workers,^{32,33} found a general reduction in the number of cytoplasmic organelles, loss of microvilli, cellular dissociation, swelling of mitochondria, and increased lipid particles and lysosome-like vesicles. The findings of Sirtori and Morano^{36,37} were quite similar to these. Moricard⁸⁸ commented that the cancer cells were in the "anarchic dispersion" of the cytoplasm, or the "cellular depolarization."

It is quite interesting that during carcinogenesis there is a definite loss of specialized cellular inclusions produced by the cell, such as secretory vesicles and cilia. In the human endometrium Nilsson et al.^{32,33} noted that apocrine-type secretion was very rare in cancer cells. In chemically induced carcinoma of the rabbit, Merriam et al., by means of histochemistry, noted a lack of glycoprotein in cancer cells.⁸⁹ Sirtori and Morano found ciliated cells in

only one exceptional case in their study of the human tumors. Oberling and Bernhard¹ in their general discussion of ultrastructure mentioned that cilia are rare in cancer cells.

As already mentioned, similar changes were seen in the chemically induced carcinoma of the rabbit endometrium. This is a stage of cytoplasmic simplification, of cellular "dedifferentiation" as Oberling and Bernhard called it.¹

Although in the end stage of carcinoma the cells assumed a highly similar appearance, the processes that led to this stage were different in spontaneous carcinogenesis from those in chemical induction of the carcinoma. During chemical induction cell injuries are considered to be the important factor, judging from the severe morphological alteration of the cells. In contrast, during spontaneous carcinogenesis one would be inclined to consider that the process of spontaneous tumor production is related to tissue senescence because of the very close resemblance between the senile cells and the carcinoma cells. Sirtori and Morano³⁷ reached a similar conclusion after their electron microscopic analysis of human materials, and stated in their monograph that "gerontology opens the door to cancerology." If the process of chemical carcinogenesis can be described as "dystrophic carcinogenesis," then that of the spontaneous carcinoma would represent an instance of "atrophy carcinogenesis."

A similar association in the stomach of tissue atrophy and carcinogenesis in which atrophic gastritis with achlorhydria and adenocarcinoma are often present concomitantly has long been known.⁸⁹ Atrophic gastritis is characterized by a marked reduction of functional parietal

and chief cells. Atrophic gastritis is frequently encountered in the senile population. It is also interesting that carcinoma of the stomach is believed to originate from the mucus-secreting cells, not from parietal or chief cells.

There is a large volume of literature concerning the association of adenomatous hyperplasia of the endometrium and/or hyperestrinism with the development of adenocarcinoma of the endometrium. In a comparative study Novak and Yui⁹⁰ observed adenomatous hyperplasia in 24% of the patients with adenocarcinoma of the endometrium. In a prospective study Taylor⁹¹ found the occurrence of malignant lesions among patients with adenomatous hyperplasia to be 2%. In an extensive retrospective approach, Hertig and his associates⁹²⁻⁹⁴ reported a high incidence of adenomatous hyperplasia and anaplasia in tissue removed 1-13 years before the diagnosis of carcinoma of the endometrium. Of the 32 cases reviewed, 13 had adenomatous hyperplasia, 13 had anaplasia, and six had both adenomatous hyperplasia and anaplasia. A review by Greene et al.⁹⁵ cited much controversial data on this subject. In a recent study Gusberg and Kaplan,⁹⁶ by the prospective approach, noted that 18% of the cases with adenomatous hyperplasia subsequently developed carcinoma of the endometrium. However, Beutler et al.,⁹⁸ in their retrospective study, did not observe a significant degree of association of adenomatous hyperplasia with carcinoma. In the postmenopausal group, 52% of the patients with carcinoma of the endometrium had shown atrophic endometrium in the previous biopsies, and in the premenopausal group 52% had normal endometrium. These authors indicated that the developmental scheme advanced by Hertig

and Sommers⁹⁵ (adenomatous hyperplasia, in-situ to invasive carcinoma) could only be observed in some premenopausal cases in which the development of carcinoma was rapid.

In experimental animals such correlation has been much less known. Greene⁹⁹ noted frequent cystic endometritis in rabbits which were sacrificed because of infertility following the toxemia of pregnancy. Since this group of rabbits had a higher incidence of carcinoma, he suggested that this type of lesion might have developed neoplastic changes if the animals had not been killed. Cotchin¹⁰⁰ stated that in the cow there is no relation between carcinoma of the endometrium and adenomatous hyperplasia. We observed certain degrees of cystic dilatation, but adenomatous hyperplasia was not present in the endometria associated with carcinoma.

The association of hyperestrogenism and carcinogenesis of the endometrium has been debated extensively for many years, and animal experiments in this field have not been conclusive. Today, it is generally accepted that a high percentage of vaginal smears obtained from postmenopausal patients with endometrial carcinoma have excessive maturation of squamous epithelial cells. This is considered to be an expression of hyperestrogenism.^{101,102} However, vaginal cornification is found to lesser extent among normal postmenopausal women without malignant lesions.¹⁰¹ Furthermore the cornified cells present in patients with endometrial carcinoma do not disappear following surgical or radiation castration in a high percentage of patients.¹⁰² Gray and Barnes¹⁰³ reviewed 176 cases with endometrial carcinoma; 27 cases had cornified vaginal smears, eight of whom had a previous history of

estrogen therapy for the past 1-14 years, and nine cases had not received estrogen therapy; 30 showed atrophic smears. The remaining 119 cases, for which smears were not available, were described as having atrophic vagina or senile vaginitis posed on clinical observations.

It might be questioned whether a persistent epithelial maturation really reflects a high estrogen level. It is well known that vaginal smears are useful only for the evaluation of relative estrogen level. If this level fluctuates or persists, the smear is difficult to evaluate. The results of some animal experiments suggest that persistent cornification cannot be considered to be proof of hyperestrinism. Estrogens injected in neonatal mice produced anovulatory animals with "sustained estrus."¹⁰⁴ Such "sustained-estrous" smears could not be altered by ovariectomy, ovariectomy and adrenalectomy, or ovariectomy and hypophysectomy, indicating that persistent epithelial cornification is due to the actual effects of estrogens. By biochemical determination of urinary estrogen secretion, Rauramo and Grönroos^{105,106} failed to detect any difference between carcinoma patients and the postmenopausal controls. On the other hand, the coexistence of feminizing ovarian tumor and endometrial carcinoma has been reported repeatedly. Mansel and Hertig¹⁰⁷ found such a combination to be 25%, whereas the combination with non-feminizing granulosa-theca cell tumor was 7%. Greene et al.⁹⁵ reviewed the previous reports and their own material and threw doubt upon such correlation. It is true that feminizing tumors do not produce endometrial carcinoma in the premenopausal period.

Carcinoma occurring in the premenopausal period is often associated

with amenorrhea,⁹⁴ and particularly with the Stein-Leventhal syndrome.¹⁰⁹ In the latter syndrome the important metabolic defects are in the conversion of androgens to estrogens within the ovarian tissue, which could explain some of the virilizing features.^{110,111} However, a large amount of Δ^4 -androstenedione and dehydroepiandrosterone accumulation was not considered to be primary, but secondary to an uncontrolled release of LH (ICSH) from the adenohypophysis.¹¹² Recently the reduction of, or lack of, LH-inhibiting substance in the urine of patients with Stein-Leventhal syndrome was reported to further substantiate this mechanism.¹¹³

In rare instances endometrial carcinoma has been associated with adrenogenital syndrome.^{114,115}

Sherman and Woolf¹¹⁶ noted that there were constantly elevated urinary LH levels in patients with endometrial carcinoma. Varga and Henriksen¹¹⁷ found such elevation in about 30% of the patients. However, Yahia¹¹⁸ did not observe a significant difference in day-to-day secretion between such patients and the normal controls.

As mentioned by Garnet and other authors,¹¹⁹ multiple constitutional abnormalities, such as hypertension, diabetes mellitus, obesity and nulliparity, are very common among patients with endometrial carcinoma. In autopsies, Sommers and Meissner¹²⁰ noted certain changes involving ovaries, adrenals, and pituitary.

In experiments with rabbits, using a combination of stilbestrol and alloxan, Sommers and Meissner¹²¹ noted hypertrophy of ovaries, adrenals, and pituitary among the animals with tumors. Greene⁹⁹ noted that certain constitutional abnormalities, such as dwarfism in the

Polish breed of rabbits, and toxemia of pregnancy, both of which showed genetic trends, were associated with significantly higher incidence of carcinoma of the endometrium. A colony of Bethesda-black rats was reported by Schulze¹²² to have a high incidence of endometrial carcinoma and also a simultaneously higher incidence of multiple endocrine tumors.

Thus all available evidence indicates that mere hyperestrinism is not likely to be the sole etiological factor of tumorigenesis of the endometrium. The most important factor seems to be multiple endocrine disturbances. Furthermore, besides the pituitary, adrenal and ovary, the neural control mechanism in the hypothalamus must play a great role in this respect. Although the number of cases was small, a recent clinical observation that adenomatous hyperplasia and adenocarcinoma of the endometrium were successfully controlled by the administration of Clomiphen is extremely interesting.¹²³

Statistically, endometrial carcinoma occurs more frequently after cessation of the menstrual cycle, and in the premenopausal age it is often associated with some menstrual disturbances. This might have some bearing on another observation: Among laboratory animals, those having an ovarian cycle with ovulation controlled by the regular neural LH-release mechanism, such as rats and mice, have a low incidence of endometrial carcinoma; whereas rabbits, without such mechanism, have a high incidence of endometrial carcinoma. In rabbits, a fluctuating level of estrogen secretion results in occasional estrus, but spontaneous LH-release or ovulation occurs only after sufficient coital stimuli.¹²⁴ Hertig and Sommers⁹⁵ implied that normal menstruation may,

in a sense, insure against the development of endometrial carcinoma, at least when it follows an ovulatory cycle. The exact mechanism of such a protective process is not known, but the phenomenon is analogous to experimental skin carcinogenesis associated with the hair growth cycle. Andreasen¹²⁴ observed that after a single application of a complete carcinogen on the mouse skin during the resting stage of hair growth cycle carcinoma developed, but if it was applied during the growing stage carcinoma failed to develop. Later experiments by Berenblum and co-workers¹²⁶ indicated that the process of initiation was much shorter in duration and not affected by the cycle. Promotion was benefited by the resting stage. In the growth phase, the carcinogens were quickly eliminated from the cells before sufficient promoting action was exerted. Naturally it is not possible to name each initiating and promoting factor in the spontaneous carcinogenesis, but the arrest of the cellular growth either at hyperplastic or atrophic level may well be the major factor in eliciting carcinoma.

In this regard, there have been only a few carcinogenic experiments on artificially anovulatory and acyclic animals. These animals were rendered anovulatory following the neonatal injection of gonadal hormones, which are known to destroy the neural regulatory mechanism of gonadal cycles. Pfeiffer¹²⁷ in 1949 treated 185 rats by testicular transplantation within a few hours of birth and obtained one adenocarcinoma after 816 days. Recently Dunn and Green,¹²⁸ by the neonatal injection of estrogens in mice, obtained squamous cell carcinomas of the uterine cervix and the vagina. It is of course improper to apply such highly artificial conditions to humans in whom aging is associated

with hormonal imbalance. Perhaps in spontaneous tumorigenesis the senility of tissue is probably more important than mere hormonal imbalance.

Beutler and others⁹⁸ noted the presence of islands of hormone-insensitive cells in the endometrium before the development of carcinoma. These were present in the lower portion of the endometrium and were considered to be the possible precursors of the cancer cells. This finding is in accord with our ultrastructural observations that at the depths of the glands there were very atrophic cells which closely resembled cancer cells. Probably during spontaneous carcinogenesis there is a certain alteration in cell population which leads to a preponderance of such less developed dormant cells. It is equally interesting that Takasugi and Bern¹²⁹ proposed that the non-estrogen dependent vaginal cornification of anovulatory mice is due to the selection of such cells which do not require estrogens for their cornification. A high percentage of the vaginal cornification in the postmenopausal women with endometrial carcinoma may well be nothing more than evidence of locally increased selective pressure in the hormone-effective genital tract.

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CHAPTER V

SUMMARY

In this investigation 267 female rabbits of the Dutch breed, 205 virgin female rats of the Bethesda Black strain, and 393 virgin female mice of the B6D2F₁ hybrid strain were subdivided into several groups: The first group of animals served as normal controls. The second group received weekly injections of diethylstilbestrol. The single dosage was 7.5 mg. in a rabbit, 1.25 mg. in a rat, and 0.12 mg. in a mouse. In the third group of animals cotton strings or tapes impregnated with 3-methylcholanthrene were surgically inserted into the uterine cavity. The average content of 3-methylcholanthrene per string was 36 mg. in a rabbit, 3 mg. in a rat, and 0.5 mg. in a mouse. The fourth group of animals received a combination of the treatment given to the second and third groups. The fifth and sixth groups were injection and string controls. The former received injections of ethyl oleate or vegetable oil, and to the latter strings coated with an equal mixture of paraffin and polyvinyl chloride were inserted into the uterine cavity.

High incidences of spontaneous adenocarcinoma in the rabbit endometrium and of spontaneous leiomyosarcoma in the mouse uterus were noted in the first group. In the animals treated with diethylstilbestrol, a reduced incidence of spontaneous tumors was noted in the rabbit and mouse. The local application of 3-methylcholanthrene as in the third group induced some adenocarcinomas in rabbits and a number

of squamous cell carcinomas in rats and mice. The simultaneous administration of diethylstilbestrol and the carcinogen enhanced the rate of development and incidence of carcinoma induction in rabbits and mice. The effect was equivocal in rats. The group of rats which were operated on only for the insertion of strings coated with paraffin-polyvinyl resin also developed a number of squamous cell carcinomas.

The tissues obtained from these animals were examined by means of light and electron microscopy. In rats and mice, squamous metaplasia developed early and was followed by dysplasia, carcinoma in situ, and carcinoma. The most striking change was the appearance of huge intramitochondrial bodies in the dysplastic endometrial cells of the mouse. The endometrial cells of the rat showed rather subtle changes. Carcinoma cells and carcinoma in situ cells were similar in their ultrastructural appearance. They resembled the basal or parabasal cells. The carcinomatous stage was characterized by nuclear and nucleolar changes and defective basement membranes.

In rabbits, severe degeneration of the cytoplasmic organelles was seen in the superficial epithelium in dysplasia; however, carcinoma in situ and carcinoma developed in the deep glandular portions of the epithelium. The cancer cells had a simplified cytoplasmic appearance. The alterations seen in the dysplastic surface epithelium often persisted without further progression, as if it were arrested.

In the spontaneous rabbit endometrial adenocarcinoma, the cancer cells similarly showed simplification of the cytoplasm. There was a very close resemblance in the cellular appearance between adenocarcinoma in situ and severe senile atrophy.

From the morphological standpoint, in the chemical carcinogenesis the cellular injuries inflicted by the chemical carcinogens seemed to play a major role. The occurrence of carcinoma was regarded as the recovery from such injurious effects by certain clones of cells that gained cellular autonomy at a lower level of differentiation. The senescence of tissue seemed an important factor in the spontaneous occurrence of tumors. Hyperestrinism or hyperplasia appeared least related to the carcinogenesis. In other words, chemical carcinogenesis is a dystrophic process whereas spontaneous carcinogenesis is an atrophic process.

Figure 1.--Normal endometrium of the rabbit (diestrus). Both surface and glandular epithelia are shown. Note small glandular spaces. Hematoxylin and eosin stain. X 160.

Figure 2.--Normal endometrium of the rabbit (diestrus). A low-power view of the surface epithelium with many non-ciliated cells. Clear spaces in the apical cytoplasm are secretory vesicles. Electron-micrograph. X 4,000.

Figure 1

Figure 2

Figure 3.--Normal endometrium of the rabbit. A luminal aspect of a ciliated cell is shown with many basal bodies. Electron-micrograph. X 11,600.

Figure 4.--Normal endometrium of the rabbit. Apical portions of the non-ciliated cells are shown with many dense bodies. The cisternae of the rough-surfaced ergastoplasm are dilated. Electron-micrograph. X 11,600.

Figure 3

Figure 4

Figure 5.--Normal endometrium of the rabbit. The luminal zone of the glandular epithelium is shown. Many multivesicular bodies and dense bodies are present. Electron-micrograph. X 17,000.

Figure 6.--The rabbit endometrium after castration. The cells are small with relatively prominent nuclei. The ciliated cells appear crowded. Hematoxylin and eosin stain. X 640.

Figure 5

Figure 6

Figure 7.--The surface epithelium of the rabbit endometrium after castration. The apical cytoplasm is reduced in amount. An arrow indicates a small cluster of glycogen granules. Electron-micrograph. X 4,000.

Figure 8.--The rabbit endometrium after castration. A marked reduction of the cytoplasm is shown in the glandular cells. Many dense bodies, about 1 μ in diameter, are shown. Electron-micrograph. X 4,000.

Figure 7

Figure 8

Figure 9.--The rabbit endometrium following estrogen administration. A marked hyperplasia of both surface and glandular epithelia is present. Both portions of the epithelium consist of ciliated and mucus-secreting cells. Osmium-fixed and Maraglas-embedded tissue. Toluidin-blue and pyronin stain. X 600.

Figure 10.--The rabbit endometrium following estrogen administration. Cilia, secretory vesicles and granules are clearly demonstrated. The vesicles are pale and limited to the apical portion of the mucus-secreting cells, while the granules are in the basal portions of the cells. Note the basal bodies of the cilia. Osmium-fixed and Maraglas embedded tissue. Toluidin-blue and pyronin stain. X 1,600.

Figure 9

Figure 10

Figure 11.--The rabbit endometrium following estrogen administration. Apical portions of ciliated and mucus-secreting cells are seen. Many electron-pale secretory vesicles fill up the cytoplasmic protrusion. Electron-micrograph. X 11,600.

Figure 12.--The rabbit endometrium following estrogen administration. The clear secretory vesicles are in a close contact with the Golgi apparatus. The arrows show dense granules as shown in Figure 10. Electron-micrograph. X 11,600.

Figure 11

Figure 12

Figure 13.--Metaplasia of the rabbit endometrium.

Methylcholanthrene effects on the surface epithelium.

A columnar epithelium has undergone metaplasia.

Hematoxylin and eosin stain. X 160.

Figure 14.--Dysplasia of the rabbit endometrium. The surface

epithelium. Nuclei and cytoplasm are both enlarged.

The cell surface is flat or convex without cilia.

The cytoplasm is dark-stained. Hematoxylin and

eosin stain. X 640.

Figure 13

Figure 14

Figure 15.--Dysplasia of the rabbit endometrium. The surface epithelium in a low-power view. The cells are round and resemble squamous cells in their construction. Besides mitochondria, the organelles are inconspicuous. Electron-micrograph. X 2,640.

Figure 16.--Dysplasia of the rabbit endometrium. The surface epithelium. A marked indentation of the cell membranes is shown. The Golgi apparatus is seen in the center. Many mitochondria show loss of the cristae or degeneration into membranous material. Electron-micrograph. X 12,000.

Figure 15

Figure 16

Figure 17.--Dysplasia of the rabbit endometrium. Besides defective mitochondria the Golgi vesicles and bizarre dense bodies are present. Many cytoplasmic fibrils are seen. Electron-micrograph. X 18,200.

Figure 18.--Carcinoma in situ of the rabbit endometrium. Many glands are present, which show some irregular appearance. Osmium-fixed and Maraglas-embedded tissue. Toluidin-blue and pyronin stain. X 600.

Figure 17

Figure 18

Figure 19.--Carcinoma in situ of the rabbit endometrium. A portion of a gland which shows large nuclei. The cytoplasm of the cells is denser than the nuclei. Osmium-fixed and Maraglas-embedded tissue. Toluidin-blue and pyronin stain. X 1,600.

Figure 20.--Carcinoma in situ of the rabbit endometrium. A portion of the cytoplasm, containing many mitochondria and vesicles, is shown. Occasional dilatation of the ergastoplasmic cisternae may be noted. Electron-micrograph. S 11,600.

Figure 19

Figure 20

Figure 21.--Adenocarcinoma of the rabbit endometrium. Many small glands are present, all lined with cuboidal cells. Some invaginations of the glands are seen. Hematoxylin and eosin stain. X 640.

Figure 22.--Adenocarcinoma of the rabbit endometrium. Irregularly arranged cells without polarity. The nucleocytoplasmic ratio is increased, and the nuclear sizes are variable. Osmium-fixed and Maraglas-embedded tissue. Toluidin-blue and pyronin stain. X 1,600.

Figure 21

Figure 22

Figure 23.--Adenocarcinoma of the rabbit endometrium. The surface epithelium. A large nucleocytoplasmic ratio and prominent nucleoli suggest a malignant nature of the cells, however, no invasiveness is noted in the surface epithelium, while the glandular portions show invasive carcinoma. Electron-micrograph. X 4,000.

Figure 24.--Adenocarcinoma of the rabbit endometrium. The glandular portion. The cells are closely packed. The cellular dissociation is seen only in the basal portions. Electron-micrograph. X 4,000.

Figure 23

Figure 24

Figure 25.--Adenocarcinoma of the rabbit endometrium. The surface epithelium. Many microvilli are present. A subluminal zone is rich in the cytoplasmic fibrils and poor in the organelles. Mitochondria are of a slightly irregular appearance. Electron-micrograph. X 13,600.

Figure 26.--Adenocarcinoma of the rabbit endometrium. The glandular epithelium. Large nucleoli, swollen mitochondria, dilated cisternae of the rough-surfaced ergastoplasm and many free ribosomes (polysomes) are present. Electron-micrograph. X 13,600.

Figure 25

Figure 26

Figure 27.--Adenocarcinoma of the rabbit endometrium. The glandular epithelium. A marked indentation of the lateral cell membranes is noted. Electron-micrograph. X 18,200.

Figure 28.--Adenocarcinoma of the rabbit endometrium. The glandular epithelium. The basal cell membranes of the cancer cells are not surrounded by a usual basement membrane, and the cytoplasmic processes are very close to the collagen fibers. Electron-micrograph. X 24,800.

Figure 27

Figure 28

Figure 29.--Adenocarcinoma of the rabbit endometrium. A basal portion of a carcinoma cell is shown, where the usual structure of the basement membrane is partially present. Some collagen fibers are seen on the right. Note a relationship between the cytoplasmic processes of the carcinoma cell and the lack of the dense zone of the basement membrane. Electron-micrograph. X 31,000.

Figure 30.--Normal endometrium of the rat. Diestrus. The cells are columnar without cilia. Glands are small and few. Hematoxylin and eosin stain. X 160.

Figure 29

Figure 30

Figure 31.--Normal endometrium of the rat. Diestrus. Surface.

The rough-surfaced ergastoplasm and mitochondria are seen. A part of a lipid droplet is seen beneath the nucleus. Electron-micrograph. X 11,600.

Figure 32.--Normal endometrium of the rat. Diestrus. The glandular portion is shown. The cytoplasmic organelles are more abundant than in the surface cells. Electron-micrograph. X 8,000.

Figure 31

Figure 32

Figure 33.--The rat endometrium after castration. The cells are short. More glandular spaces are seen per a microscopic field. Note the absence of the leukocytes. Hematoxylin and eosin stain. X 160.

Figure 34.--The rat endometrium after castration. A surface cell. A few small microvilli are present. The Golgi apparatus is prominent, and many lipid droplets are seen. Electron-micrograph. X 8,000.

Figure 33

Figure 34

Figure 35.--The rat endometrium after castration. The rough-surfaced ergastoplasm is well-developed and the cisternae are narrow. A few dense bodies are seen. X 11,600.

Figure 36.--The rat endometrium following estrogen administration. A low-power view of the surface epithelium to demonstrate tall hyperplastic cells. Electron-micrograph. X 2,640.

Figure 35

Figure 36

Figure 37.--The rat endometrium following estrogen administration.

The surface epithelium. Many dense bodies are present.

The Golgi apparatus is well developed. The ergastoplasmic cisternae are distended. Electron-micrograph.

X 11,600.

Figure 38.--The rat endometrium following estrogen administration.

The glandular portion consists of many cells with abundant cytoplasmic organelles. Many leukocytes are present among the epithelial cells. Electron-micrograph.

X 4,000.

Figure 37

Figure 38

Figure 39.--Metaplasia of the rat endometrium. Dense keratinization is present. The basal cell zone consists of one or two layers of pale-staining cells. Osmium-fixed and Maraglas-embedded tissue. Toluidin-blue and pyronin stain. X 600.

Figure 40.--Metaplasia of the rat endometrium. A portion of the spindle-shaped prickle-cell layer is shown. Desmosomes are very prominent. Electron-micrograph. X 3,600.

Figure 39

Figure 40

Figure 41.--Metaplasia of the rat endometrium. Keratin fibrils are very abundant. Among the bundles of keratin fibrils, some dense bodies and mitochondria are present. The dense bodies are either keratohyalin granules or membrane-surrounded granules. Electron-micrograph. X 12,000.

Figure 42.--Metaplasia of the rat endometrium. A portion of the subluminal layer is shown. Many keratin fibers and some vesicles are present. Note many desmosomes. Electron-micrograph. X 31,400.

Figure 41

Figure 42

Figure 43.--Metaplasia of the rat endometrium. A portion of the prickle-cell layer showing keratin fibrils and desmosomes. Mitochondria do not show abnormality. Electron-micrograph. X 31,400.

Figure 44.--Dysplasia of the rat endometrium. Less keratinization is present. The cells show enlarged nucleoli. Osmium-fixed and Maraglas-embedded tissue. Toluidin-blue and pyronin stain. X 1,600.

Figure 43

Figure 44

Figure 45.--Dysplasia of the rat endometrium. The luminal layer is shown. Those spindle-shaped cells are poor in the cytoplasmic organelles. Some clear spaces are actually swollen mitochondria or dilated ergastoplasmic cisternae. Electron-micrograph. X 5,600.

Figure 46.--Dysplasia of the rat endometrium. A paucity of the cytoplasmic organelles is shown. There are few ribosomes and markedly swollen mitochondria. Abundant keratin fibrils are present. Electron-micrograph. X 12,000.

Figure 45

Figure 46

Figure 47.--Carcinoma in situ of the rat endometrium. A marked hyperplasia of the basal and parabasal cell zones is noted. Nuclei are enlarged and show irregular size. Nucleoli are very prominent. Osmium-fixed and Maraglas-embedded tissue. Toluidin-blue and pyronin stain. X 600.

Figure 48.--Carcinoma in situ of the rat endometrium. The cytoplasm is darker than the nuclei. The cytoplasmic organelles are more abundant than the dysplastic stage. Electron-micrograph. X 3,600.

Figure 47

Figure 48

Figure 49.--Carcinoma in situ of the rat endometrium. A portion of the cytoplasm containing many keratin fibrils, membrane-bound granules (secondary to the keratin formation), scattered glycogen granules and ribosomes. Electron-micrograph. X 31,400.

Figure 50.--Carcinoma in situ of the rat endometrium. Dense condensations are seen in the nucleus. On the left is a part of enlarged pars amorpha of a nucleolus. Electron-micrograph. X 20,800.

Figure 49

Figure 50

Figure 51.--Carcinoma in situ of the rat endometrium. A basal cell. Keratin fibrils are condensed in bundles. A peculiar indentation of the basal cell membrane is noted. Electron-micrograph. X 20,800.

Figure 52.--Squamous cell carcinoma of the rat. A large tumor is noted in the proximal portion of the uterus. A distal segment is shown by an arrow.

Figure 51

Figure 52

Figure 53.--Squamous cell carcinoma of the rat. The cut surface shows partly necrotic tumor. The arrow indicates a space where a 3-methylcholanthrene-impregnated string was present.

Figure 54.--Squamous cell carcinoma of the rat endometrium. Keratinization is very prominent. Hematoxylin and eosin stain. X 160.

Figure 53

Figure 54

Figure 55.--Squamous cell carcinoma of the rat endometrium.

An invasive portion of the carcinoma comprised of many disoriented basal cells is shown. Many leukocytes are present. Hematoxylin and eosin stain. X 160.

Figure 56.--Squamous cell carcinoma of the rat endometrium.

Moderate enlargement of nuclei and nucleoli is shown. The desmosomes seem also enlarged. Electron-micrograph. X 3,600.

Figure 55

Figure 56

Figure 57.--Squamous cell carcinoma of the rat endometrium.

Several dense particles are seen in both nucleus and cytoplasm. Those in the nucleus are condensed chromatin, and those in the cytoplasm represent large keratohyalin granules. Electron-micrograph. X 12,000.

Figure 58.--Squamous cell carcinoma of the rat endometrium.

Many mitochondria and ribosomes are present. The keratin fibrils are seen in bundles in the peripheral portions of the cytoplasm. Electron-micrograph. X 12,000.

Figure 57

Figure 58

Figure 59.--Normal endometrium of the mouse. Diestrus. Many glands are present, which are lined with paler-staining cells. Hematoxylin and eosin stain. X 160.

Figure 60.--Normal endometrium of the mouse. A surface cell during diestrus. The cytoplasm is edematous. Many lipid droplets are present in the subnuclear region. Electron-micrograph. X 11,600.

Figure 59

Figure 60

Figure 61.--Normal endometrium of the mouse. The basal portion of the surface epithelial cells during diestrus. Electron-micrograph. X 11,600.

Figure 62.--Normal endometrium of the mouse. A glandular portion is shown. Electron-micrograph. X 4,000.

Figure 61

Figure 62

Figure 63.--Normal endometrium of the mouse. The apical portions of the glandular cells are shown. There are many cytoplasmic organelles including a few dense bodies. Electron-micrograph. X 11,600.

Figure 64.--Normal endometrium of the mouse. In some glands very small amount of secretion can be recognized. Electron-micrograph. X 8,000.

Figure 63

Figure 64

Figure 65.--The mouse endometrium after castration. The cells are slightly shorter. Relatively as many glands are seen on section as in a castrated rat. Hematoxylin and eosin stain. X 160.

Figure 66.--The mouse endometrium after castration. The luminal portions of the surface epithelium are shown. The Golgi apparatus and mitochondria are noted, but the ergastoplasm is inconspicuous. Electron-micrograph. X 14,000.

Figure 65

Figure 66

Figure 67.--The mouse endometrium after castration. The basal aspects of the surface epithelium. Note an abundance of the lipid droplets. Electron-micrograph. X 11,600.

Figure 68.--The mouse endometrium after castration. The luminal portion of the glandular cells are shown. The cytoplasmic organelles are more abundant. Note a large dense body, probably autophagic lysosomes. Electron-micrograph. X 11,600.

Figure 67

Figure 68

Figure 69.--The mouse uterus following estrogen administration.

A marked distention of the uterine horns is noted.

Figure 70.--The mouse endometrium following estrogen administration.

The cells are tall and the epithelium looks crowded.

Many vacuoles are seen in the cells. Hematoxylin and eosin stain. X 160.

Figure 69

Figure 70

Figure 71.--The mouse endometrium following the estrogen administration. The apical cytoplasm of the glandular epithelial cells contains numerous small dense particles, secretory granules. The lower half of the apical cytoplasm shows well developed Golgi apparatus and many larger dense bodies. The lumen is dark and contains many small particles. Electron-micrograph. X 4,000.

Figure 72.--The mouse endometrium following estrogen administration. The apical portion of the surface epithelial cells contains several large membrane-surrounded bodies as shown here. But small dense particles seen in the glandular cells are rarely present. Electron-micrograph. X 31,400.

Figure 71

Figure 72

Figure 73.--Metaplasia of the mouse endometrium. Early metaplasia is noted in the surface epithelium. Dark nuclei are those of the invading leukocytes. Hematoxylin and eosin stain. X 640.

Figure 74.--Metaplasia of the mouse endometrium. The surface epithelium shows a typical structure of the squamous epithelium. Osmium-fixed and Maraglas-embedded tissue. Toluidin-blue and pyronin stain. X 600.

Figure 73

Figure 74

Figure 75.--Metaplasia of the mouse endometrium. Two weeks after the insertion of a carcinogen-impregnated string, the glandular epithelial cells still show the secretory activity under the estrogen effects. The apical portion of their cytoplasm is shown. Electron-micrograph. X 24,800.

Figure 76.--Metaplasia of the mouse endometrium. Four weeks after the insertion, however, no secretory activity is seen. Electron-micrograph. X 13,600.

Figure 75

Figure 76

Figure 77.--Metaplasia of the mouse endometrium. Keratin fibrils are condensed in the peripheral portion of the cells in the upper portion of the metaplastic epithelium. Many leukocytes are present. Electron-micrograph. X 4,000.

Figure 78.--Metaplasia of the mouse endometrium. The luminal layer of a non-keratinized cell. The cytoplasmic organelles are reduced in number. Microvilli are present on the surface. Electron-micrograph. X 13,600.

Figure 77

Figure 78

Figure 79.--Metaplasia of the mouse endometrium. A basal cell shows more mitochondria and ribosomes and less keratin fibrils. The matrix of the mitochondria is electron-pale and no abnormal inclusion is present. Electron-micrograph. X 24,800.

Figure 80.--Dysplasia of the mouse endometrium. Besides a marked pyometra, the surface epithelium crowded with the elongated cell is noted. A portion of a cotton string which has been inserted is seen on the right surrounded by pus-cells. Hematoxylin and eosin stain. X 160.

Figure 79

Figure 80

Figure 81.--Dysplasia of the mouse endometrium. A marked increase in the basal cells. Nuclei are vesicular and large with prominent nucleoli. Osmium-fixed and Maraglas-embedded tissue. Toluidin-blue and pyronin stain. X 600.

Figure 82.--Dysplasia of the mouse endometrium. The middle portion of the epithelium shows typical prickly cells with large nuclei. Several dense bodies are seen in the cytoplasm. Electron-micrograph. X 4,000.

Figure 81

Figure 82

Figure 83.--Dysplasia of the mouse endometrium. The luminal layer of the epithelium shows very scanty ergastoplasm, degenerating mitochondria and a few bizarre bodies. The emptiness of the cytoplasm is obvious. Electron-micrograph. X 13,600.

Figure 84.--Dysplasia of the mouse endometrium. Numerous huge intramitochondrial bodies are present. They are homogeneous in the electron-density. They appear to be surrounded by the cristae. Note glycogen granules. Electron-micrograph. X 24,800.

Figure 83

Figure 84

Figure 85.--Dysplasia of the mouse endometrium. The huge intramitochondrial bodies are surrounded by the cristae. Electron-micrograph. X 30,400.

Figure 86.--Carcinoma in situ of the mouse endometrium. A marked crowding of the elongated cells is seen. The cells resemble the basal cells seen in the metaplasia. Still many inflammatory cells are present. Hematoxylin and eosin stain. X 640.

Figure 85

Figure 86

Figure 87.--Carcinoma in situ of the mouse endometrium. An elongation of abnormal epithelium is shown. A marked cellular irregularity is noted. Hematoxylin and eosin stain. X 640.

Figure 88.--Carcinoma in situ of the mouse endometrium. A marked indentation of the cell membranes with many desmosomes is shown. Abundant keratin fibrils are present between the nucleus and the cell membrane. Electron-micrograph. X 13,600.

Figure 87

Figure 88

Figure 89.--Carcinoma of the mouse endometrium. A part of the nucleolus is seen on the left. The cytoplasm contains a moderate amount of keratin fibrils, free ribosomes and polysomes. The mitochondria show very small intra-mitochondrial bodies. The cristae mitochondriales show the usual arrangement. Electron-micrograph. X 24,800.

Figure 90.--Squamous cell carcinoma of the mouse endometrium. The invasive islands of carcinoma cells are shown. The secondary inflammation is also noted. Hematoxylin and eosin stain. X 160.

Figure 89

Figure 90

Figure 91.--Adenocarcinoma of the mouse endometrium. One of the rare instances in which adenocarcinomas developed. Hematoxylin and eosin stain. X 160.

Figure 92.--Squamous cell carcinoma of the mouse endometrium. The cellular irregularity is shown. Some cells are in mitoses. Hematoxylin and eosin stain. X 640.

Figure 91

Figure 92

Figure 93.--Adenocarcinoma of the mouse endometrium. The glandular epithelium shows a distinct malignant transformation. Hematoxylin and eosin stain. X 640.

Figure 94.--Squamous cell carcinoma of the mouse endometrium. The nuclei are markedly enlarged, so are the nucleoli. The nucleolonema can be reorganized as dense strands. Electron-micrograph. X 8,000.

Figure 93

Figure 94

Figure 95.--Squamous cell carcinoma of the mouse endometrium. The basal portion of a cancer cell is shown. Above an arrow there is a typical triple zone formation of the basement membrane seen with some hemidesmosomes. Below it, however, no definite structure can be identified. There are many cytoplasmic processes in place. Electron-micrograph. X 11,600.

Figure 96.--Squamous cell carcinoma of the mouse endometrium. The carcinoma cells are present on the left. The cell membrane on the right is that of a fibrocyte. Note the absence of the usual dense outer zone of the basement membrane. Only very slight condensation of amorphous material is noted. Electron-micrograph. X 40,000.

Figure 95

Figure 96

Figure 97.--Spontaneous leiomyosarcoma of the uterus in a mouse.

One of the uterine horns is enlarged because of the tumor. The serosal surface of the small intestine shows some tumor implants.

Figure 98.--Senile atrophy of the rabbit endometrium. The surface epithelium is thin. The glands are small and occasionally distended. Hematoxylin and eosin stain. X 100.

Figure 97

Figure 98

Figure 99.--Senile atrophy of the rabbit endometrium. The deepest portion of the gland consists of small cells with little cytoplasm. The cytoplasmic organelles are poorly developed. Electron-micrograph. X 8,000.

Figure 100.--Senile atrophy of the rabbit endometrium. The glandular cells in the slightly higher portion. No microvilli are present. Only small numbers of the organelles are seen. No secretion or cilia are present. Electron-micrograph. X 11,600.

Figure 99

Figure 100

Figure 101.--Senile atrophy of the rabbit endometrium. The cells lining a cystic space, as seen in the Figure 100. The subluminal zone is dense and structureless. Some mitochondria, the Golgi apparatus and ergastoplasm are noted. Electron-micrograph. X 11,600.

Figure 102.--Cystic hyperplasia of the rabbit endometrium. A portion of cystic gland and the overlying surface epithelium are shown. There are some cilia in the lining cells. Osmium-fixed and Maraglas-embedded tissue. Toluidin-blue and pyronin stain. X 600.

Figure 101

Figure 102

Figure 103.--Cystic hyperplasia of the rabbit endometrium. The cells show a moderate degree of development of the organelles. X 11,600.

Figure 104.--Cystic hyperplasia of the rabbit endometrium. The non-ciliated cells in the lining of the cystic space may not show active secretion, but contain many secretory granules, electron-dense and single-membrane-surrounded, possibly representing the storage form of mucus, on the right. Electron-micrograph. X 11,600.

Figure 103

Figure 104

Figure 105.--Glandular hyperplasia of the rabbit endometrium. A marked hyperplasia of the glands is present. The lining cells are all in the upright position and contain a moderate amount of cytoplasm. Many cilia are seen. There is no abnormality of nuclei with regard to size or polarity. Osmium-fixed and Maraglas-embedded tissue. Toluidin-blue and pyronin stain. X 100.

Figure 106.--Glandular hyperplasia of the rabbit endometrium. The cells are very regular, and long microvilli are seen. A few dense bodies are present in the cytoplasm. The nucleoli are prominent but are not enlarged. Electron-micrograph. X 2,640.

Figure 105

Figure 106

Figure 107.--Glandular hyperplasia of the rabbit endometrium. The apical portion of the cells is shown. A few secretory vesicles are present immediately beneath the cell membrane. The cytoplasmic organelles are abundant. Electron-micrograph. X 11,600.

Figure 108.--Carcinoma in situ of the rabbit endometrium. (Spontaneous.) A number of irregular glands is shown. The lining cells are cuboidal or almost flat. The staining characteristics of the cytoplasm are very variable. On the left, a large space lined by very atrophic epithelial cells indicates a relationship between atrophy and in situ change. Osmium-fixed and Maraglas-embedded tissue. Toluidin-blue and pyronin stain. X 100.

Figure 107

Figure 108

Figure 109.--Carcinoma in situ of the rabbit endometrium. Portions of cystic spaces are shown. The cells show darker cytoplasm than the nuclei. The glands are very close. The lumens contain many degenerating cancer cells. Electron-micrograph. X 2,640.

Figure 110.--Carcinoma in situ of the rabbit endometrium. Cuboidal epithelial cells with short microvilli. A few mitochondria and some cisternae of the rough ergastoplasm are present. Note the increased nucleocytoplasmic ratio. X 12,000.

Figure 109

Figure 110

Figure 111.--Carcinoma in situ of the rabbit endometrium.

(Spontaneous.) An increased nucleocytoplasmic ratio. Many dense bodies probably representing lysosomes are present. Electron-micrograph. X 13,600.

Figure 112.--Adenocarcinoma of the rabbit endometrium.

(Spontaneous.) The uterine horns are distended and nodular, due to carcinomatous tissue growth.

Figure 111

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Figure 112

Figure 113.--Adenocarcinoma of the rabbit endometrium. (Spontaneous.)

The uterine cavity has been opened. The tumor has a polypous appearance with central ulceration. Note a marked thinning of the uterine wall.

Figure 114.--Adenocarcinoma of the rabbit endometrium. (Spontaneous.)

Many irregular glands are present. Note the intact surface epithelium. The cells are flattened but there is no sign of malignant change. Hematoxylin and eosin stain. X 100.

Figure 113

Figure 114

Figure 115.--Adenocarcinoma of the rabbit endometrium. (Spontaneous.)

A single tumor nodule consisting of small cells is shown. Osmium-fixed and Maraglas-embedded tissue. Toluidin-blue and pyronin stain. X 600.

Figure 116.--Adenocarcinoma of the rabbit endometrium. (Spontaneous.)

Many cells are shown, some of which are electron-dense. Some cellular dissociation is present. A marked increase in the nucleocytoplasmic ratio is seen. In spite of different density, the cells are fairly uniform in their appearance in a low-power electron microscope field. X 2,640.

Figure 115

Figure 116

Figure 117.--Adenocarcinoma of the rabbit endometrium. (Spontaneous.)

Carcinoma cells have large nuclei and very simplified cytoplasmic structure. Individual organelles, however, retain their normal appearance. The lumen contains cellular debris. Electron-micrograph. X 11,600.

Figure 118.--Adenocarcinoma of the rabbit endometrium. (Spontaneous.)

The basal aspect of the carcinoma is shown. A condensation of the reticulin fibers is noted under the basement membrane. An arrow indicates a peculiar cytoplasmic process formation. Electron-micrograph. X 11,600.

Figure 117

Figure 118

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