THE DEVELOPMENT OF TUMORS IN FEMALE MICE TREATED WITH 1:2:5:6-DIBENZANTHRAcene AND THEELIN

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The chemical similarity of the female sex hormone and the carcinogenic hydrocarbons, and the discovery that some of the latter are estrogenic, have given rise to much speculation and experimentation. One such study is reported here.

METHODS

A colony of 150 adolescent female mice was divided into two lots, of which one was spayed and the other not. These two lots were subdivided into halves, one of which was painted with 1:2:5:6-dibenzanthracene and the other with 1:2:5:6-dibenzanthracene and theelin. Four groups were thus obtained:

Group I: Normal females painted with 1:2:5:6-dibenzanthracene
Group II: Normal females painted with 1:2:5:6-dibenzanthracene and theelin
Group III: Spayed females painted with 1:2:5:6-dibenzanthracene
Group IV: Spayed females painted with 1:2:5:6-dibenzanthracene and theelin

A 0.3 per cent solution of 1:2:5:6-dibenzanthracene in benzene, prepared from yellow crystals purchased from the Eastman Kodak Company, was applied to the skin at the nape of the neck twice a week with a camels' hair brush dipped once into the solution. Six weeks after the beginning of the 1:2:5:6-dibenzanthracene treatment, application of theelin was begun, this interval being allowed because of the more rapid proliferative action of the latter substance. A 0.1 per cent solution of pure crystalline theelin in benzene was applied twice a week in the same manner as the dibenzanthracene. This dose is estimated at about 125 rat units. To reduce the hazard of benzene poisoning, the 1:2:5:6-dibenzanthracene for Groups II and IV was subsequently put into solution with the theelin. After the development of pyometra (see below), the interval between applications of theelin was extended to a week. Treatment was continued throughout the life of the animal. Vaginal smears were taken twice a week throughout the experiment.

The mice used were of an unpedigreed albino stock. Their exact source could not be traced. The laboratory, however, has purchased from only a few sources for a number of years. The animals from two sources were

1 The word theelin is used here as equivalent to estrone. The paper by Gardner, Smith, Strong, and Allen (J. A. M. A. 107: 656, 1936), published since the submission of this paper (July 25, 1936), establishes the identity of the terms.

2 The theelin was generously supplied by Parke, Davis & Co., through the courtesy of Dr. Oliver Kamm.
known to be of a non-tumor bearing strain, and it is not likely that the others were of tumor-bearing strains. That no carcinomas of the breast developed in Group I would indicate that those occurring in the other groups were not spontaneous.

The animals were autopsied at death or sacrificed when moribund. What sections were taken for microscopy depended upon the gross findings.

RESULTS

The relatively simple objective of the experiment, to compare the incidence of tumors, anticipated to be skin and genital carcinomas, in spayed and normal female mice receiving $1:2:5:6$-dibenzanthracene and $1:2:5:6$-dibenzanthracene with theelin, was complicated by the production of other tumors and lesions. Papillomas of the skin, uterus, vagina, lung, kidney, stomach, colon, and bladder occurred, as well as cutaneous horns, sebaceous cysts, adenomas of the lung and maxillary sinus, cystadenomas of the mei-

![Chart I](chart.png)

**Chart I. Distribution of Malignant Tumors According to Group and Time**

bomian gland and of the breast, cysts of the ovary and para-urethral glands, carcinomas of skin, breast, ureters, lung, stomach and colon, myofibroma, hemangioma, lymphoblastoma and thymoma. There were many cases of generalized lymphadenitis, sometimes with an infiltration of monocytic cells in the liver and kidney suggestive of leukemia. A diagnosis of leukemia was not warranted, however, because of failure to study the blood and the complicating use of benzene and the development of abscesses. It is, nevertheless, a consideration to be borne in mind by future workers.

**Skin** (Figs. 1-4): Skin papillomas were practically universal, as is common when the skin is painted with a carcinogenic substance. No systematic survey was made for small benign tumors. The occurrence of papillomas in the viscera seems reasonably explained as a systemic effect of the carcinogenic substances. There were many sebaceous cysts; and many of the skin carcinomas arose in such cysts. A few cysts were noted in other organs. The usual phenomenon of alopecia early in the course of the painting followed by reappearance of hair and the development of papillomas before the appearance of malignancy was noted in this experiment. The second crop of papillomas
did not occur except in association with the return of hair growth. Sections at this stage show the hair follicles to be numerous and crowded. The benign precancerous skin lesions were similar to those observed in human dermatological practice.

After the appearance of the secondary papillomas of the skin, the mice developed many abscesses, especially about the painted area and eyes. How much of this was due to the ulceration of tumors, to the leukocytic invasion accompanying metaplasia, and to secondary infection, could not be determined. It is quite likely that some carcinomas of the skin were missed because of ulceration. Carcinoma was diagnosed only microscopically, and in this respect understatement was preferred to overstatement where there was any doubt. Because of the varieties of lesions developing and the constantly changing population of the groups, no real statistics were possible; numbers and percentages are stated only to corroborate the trends observed in this study.

Many secondary papillomas developed about the face and a few on other parts of the body. Those of the face were noticeable for the distribution, which was the same as that observed in human basal-cell carcinomas, i.e., about the eyes, ears, and nose. Perhaps because the face was so readily scratched, these lesions were almost universally subject to suppuration. In the painted area some of the tumors developed the pearly rolled edge and central ulceration typical of skin carcinomas in man. McFarland (1) states that basal-cell carcinomas have never been produced experimentally. Woglom (2) noted that tar tumors have the gross appearance of basal-cell carcinomas. Yamagiwa and Itchikawa (3) found the majority of tar cancers
in their rabbits to be of the rodent ulcer type. In our animals we found basal-cell carcinoma, squamous-cell carcinoma, and mixed types. Squamous-cell carcinoma, however, was predominant. While basal-cell carcinomas were thus produced experimentally, they were not made the subject of an individual experimental study, but were considered only as a part of the whole problem of carcinogenesis.

The following table summarizes the incidence of carcinoma of the skin in our series.

<table>
<thead>
<tr>
<th></th>
<th>Group I</th>
<th>Group II</th>
<th>Group III</th>
<th>Group IV</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of animals when first carcinoma of the skin was recorded</td>
<td>18</td>
<td>3</td>
<td>25</td>
<td>15</td>
</tr>
<tr>
<td>Number of carcinomas of the skin</td>
<td>9</td>
<td>2</td>
<td>14</td>
<td>1</td>
</tr>
<tr>
<td>Percentage of carcinomas of the skin</td>
<td>50</td>
<td>66</td>
<td>56</td>
<td>6</td>
</tr>
</tbody>
</table>

The actual numbers of skin carcinomas in Groups II and IV were small because of the mortality from benzene poisoning, pyometra, and carcinoma of the breast before time for the development of carcinoma of the skin. Chart 1 shows the distribution of the most frequent types of malignant tumors in this experiment. In two of the cases of carcinoma of the skin there were metastases to the axillary lymph nodes, and in another case metastases to the lungs. The carcinomas of the breast occurred earlier in the groups receiving theelin than in Group III. The time of development for the skin and breast carcinomas is similar to that for spontaneous tumors of these types, and the findings are in accord with Branch's (4) report of a lower incidence of skin
Fig. 3. Skin: Papillomas

Fig. 4. Skin: Sebaceous Cyst with Intracystic Papillomas
carcinomas in mice of a spontaneous mammary carcinoma strain than in a non-tumor-bearing strain.

The data of this experiment do not warrant a definite conclusion, but our opinion is that the incidence of carcinoma of the skin in females, as produced by 1:2:5:6-dibenzanthracene, was not intrinsically affected by either spaying or the use of theelin. Burrows (5) and Cramer (6) have painted mice with estrin for long periods without producing carcinomas of the skin. Cramer says: "Estrin is absorbed by the unbroken skin without any carcinogenic effect."

**FIG. 5. BREAST: HYPERPLASIA OF NIPPLE, DUCTS, ALVEOLI, AND LYMPH NODE**

*Breast (Figs. 5-16):* Seventeen animals developed carcinoma of the breast. The group distribution was as follows:

<table>
<thead>
<tr>
<th>Group</th>
<th>Animals</th>
<th>Breast Carcinomas</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>25</td>
<td>0</td>
</tr>
<tr>
<td>II</td>
<td>14</td>
<td>6 (43%)</td>
</tr>
<tr>
<td>III</td>
<td>37</td>
<td>4 (11%)</td>
</tr>
<tr>
<td>IV</td>
<td>15</td>
<td>7 (43%)</td>
</tr>
</tbody>
</table>

Thirteen of the tumors were of the hemorrhagic type and were readily identified grossly. Figs. 5-7 show the lymph node within the breast to be hyperplastic and highly vascular. This appears to be an early stage in the angiomatous development of such tumors (Fig. 12). Ten of the breast carcinomas were observed clinically before the sixth month of treatment, and

*Throughout this paper the time from the beginning of treatment refers to the beginning of treatment with 1:2:5:6-dibenzanthracene unless otherwise stated.*
the last to occur was noted a month before death. Eleven of the carcinomas of the breast were on the shoulder; the others in the inguinal region. This would indicate that the areas adjacent to and draining the lymphatics from the painted area received proportionally larger doses than were received sys-

![Image](image-url)

**Figs. 6 and 7. Breast: Advanced Hyperplasia with Increasing Vascularity**

temically, and that the biological response was somewhat proportional to the dosage. Seven breast tumors metastasized: 5 to the lung, 1 to the axillary lymph nodes, and 1 to both lungs and lymph nodes. In 4 instances the mammary carcinomas were multiple.
After six weeks of treatment with theelin, the nipples of the mice were conspicuously enlarged. The breasts were not sectioned routinely. In 9 cases of carcinoma of the breast, sections of the other breast were taken; all showed hyperplasia. The acini were numerous and large; the lumina con-
tained a homogeneous pink-staining substance. The picture resembled a pre-lactating breast and in some cases was associated with adenoma, ductal hyperplasia, cysts, and fibrosis (Figs. 9–16). In 2 cases of carcinoma of the breast, typical "blue-domed" cysts were observed grossly. A number of breast sections from Groups II and IV were included in the sections of the vaginal orifice, because of the occurrence of perineal mammæ. These breasts showed the same hyperplastic changes.

Multiple Tumors: The tendency for both spontaneous and induced tumors to be multiple has been previously reported. In several of our animals this was strikingly illustrated. As many as 12 carcinomas of the skin occurred in one mouse. In the chart multiple carcinomas of the same type are ignored. While no quantitative analysis of the benign tumors has been attempted, the general impression is that they were more numerous in animals bearing malignant tumors than in others, and that a causal relationship exists between hyperplastic, benign, and malignant growth.

It would be expected that the groups receiving theelin would show stimulation in both breast and uterus. In one of the 4 spayed mice with carcinoma of the breast following treatment with 1:2:5:6-dibenzanthracene only, the uterus was not sectioned. In the other three cases the uterus was moderately hyperplastic. Of the 3 cases of carcinoma of the cervix observed, 2 were in mice with mammary carcinomas. The breast tumor was the cause of death in one instance. In the mouse with advanced carcinoma of the uterus the breast carcinoma was large. In the remaining case of carcinoma of the uterus,
FIG. 9. BREAST: HYPERPLASIA, CYST, AND PAPILLOMA

FIG. 10. BREAST: ADENOMA
cystic hyperplasia of the breast was present. In addition to hyperplasia of the uterus and vagina, which was universal in Groups II and IV, in association with carcinoma of the breast, a large polyp of the vagina was noted in one case (Fig. 31); in another case there was a nodular cervix; in 2 cases metaplasia of the bladder; in 4 cases cystic hyperplasia of the para-urethral glands; in 6 cases pyometra; in 1 case hydronephrosis; in 1 case pyonephrosis.

The incidence of pyometra in carcinomas of the breast has been referred to. Two of the mice with carcinoma of the uterus also had pyometra. In one this occurred as a circumscribed sac of inspissated pus at the tip of one of the uterine horns. The presence of pyometra in association with carcinoma of the primary and secondary sex organs, and the number of cases in the literature of metaplasia without carcinoma, suggest that large doses of theelin over

![Image](https://example.com/image.jpg)

**Fig. 11. Breast: Intraductal Papillomas and Atypical Epithelium**

a long period are required to produce carcinoma in the uterus. A dose of theelin large enough to produce fatal pyometra, however, excludes carcinogenesis. Our dose of theelin was too large; the optimum dose is perhaps less than we used and greater than those reported by others.

That 125 rat units of theelin would be periodically released from the ovary of a mouse is obviously improbable. Is this, or an effect similar to it, within the range of spontaneous pathology? Gardner, Strong, and Smith (7) reported the spontaneous occurrence of an adenoma of the pituitary, granulosa-cell tumors of the ovaries, multiple carcinomas of the breast, and cystic hyperplasia of the uterus in one mouse. Slye (8) reported a "glandular carcinoma" filling the vagina, which she believed might have arisen from the cervix, though its origin was not established. The same mouse had a carcinoma of the breast which was histologically different from the vaginal tumor.
This case is strikingly like that in our series in which a large carcinoma of the cervix was associated with carcinoma of the breast.

The mouse has five times as many mammae as man, has an uninhibited sex life, is frequently inbred, and is not preserved in senility. For these reasons we should not expect a close analogy between the two species in the occurrence of tumors of the generative organs. Schreiner (9) reported that of 307 cases of multiple carcinomas, 29 involved the generative organs; 15 of these were carcinomas of the breast, associated 4 times with carcinoma of the ovary, 4 times with carcinoma of the fundus of the uterus, 4 times with carcinoma of the cervix, and once each with carcinoma of the vagina, bladder, and vulva. Hellendall (10) reported a case of carcinoma of the breast and of the cervix in the same patient and cited 10 other cases in the literature. Meigs (11) found that 42 per cent of 250 multiple pelvic tumors were associated with other benign tumors, and among 25 malignant pelvic tumors associated with other tumors, in 56 per cent the coexisting malignancy was in the breast. Ingleby (12) reported 3 cases of coexisting cystic hyperplasia of the breast and of the uterus. Ewing (13) says: “Tumors have been observed simultaneously in the uterus, ovaries, and breast, a combination suggesting the influence of the functional relation existing between these organs.”

**Uterus** (Figs. 17–30): Hyperplasia of the uterus and vagina was universal in our groups of mice receiving theelin. Cystic hyperplasia and papillomas occurred frequently. Squamous-cell metaplasia in the uterus was common. Epidermoid carcinoma of the cervix was produced three times in the mice treated with theelin and 1:2:5:6-dibenzanthracene (Figs. 18–20): twice in spayed animals and once in a normal mouse. In one of the spayed mice the tumor appeared in the sixth month; the other tumors occurred in the tenth month.

On the basis of the animals in the various groups at the time of autopsy of
the first animal with tumor, the percentage for Group IV is 20 per cent; for Group II 100 per cent. If, however, we evaluate the mortality from pyometra, carcinoma of the breast, and benzene poisoning, 20 per cent is probably an underestimate and 100 per cent an overestimate. It is interesting that in Groups II and IV, the last survivor developed carcinoma of the uterus. In Group II this animal outlived its companions ten weeks. In Group IV the last survivor outlived its companions by only two weeks. The last mouse in Group IV also had a polyp of the vagina.

Microscopically, the 3 carcinomas of the uterus were all epidermoid. Two were early and their origin in the cervix is shown definitely in the accompanying illustrations (Figs. 21–29). In one instance, that in which the tumor attained a large size, a slightly oblique section at the level of the internal os shows a large mass of carcinoma which appears to arise from the left cervical canal, which it has destroyed. The uterine horns, ureters, and bladder retain their structure. The carcinoma is also in and attached to the vaginal wall. The deduction is that this tumor arose in the cervix, which it has destroyed. The question of its arising in the vagina and extending upward is not excluded, although 40 sections from 4 blocks were studied.

Woglom (14) reported one case of spontaneous carcinoma of the uterus in a mouse. Slye in 39,000 autopsies found no established case of spontaneous carcinoma of the uterus in mice. Her probable case has been discussed in connection with multiple tumors.

Carcinoma of the uterus has been produced experimentally 4 times. In
Fig. 14. Breast: Alveolar Hyperplasia with Retained Secretion

Fig. 15. Breast: Alveolar Carcinoma
1934 Pierson (15) produced a carcinoma of the uterus in one of 25 rabbits treated with various combinations of tar, folliculin, luteinogen, and castration. Of the 2 last survivors, living thirteen months after the beginning of the treatment, one developed a carcinoma and the other an adenoma of the uterus. These two animals were castrates treated with tar and folliculin. The rabbit with the carcinoma of the uterus had also a polyp of the vagina. This and our series are the only carcinomas of the uterus produced without local trauma.

Eight weeks after treatment with theelin was begun, the first animal died of pyometra. Treatment was omitted during one of these weeks because of the mortality from benzene poisoning. Four weeks later the interval between theelin applications was reduced by half. At the time of the first death from pyometra, there were 22 mice in Group II and 16 in Group IV. Eleven mice in Group II died of pyometra, and 12 in Group IV. In a few instances death was due to pyonephrosis, but as this is probably secondary to obstruction of the large genital organs, it is not considered separately. The occurrence of pyometra confirms Burrows' report not only as to its production, but as to the time and dosage involved. Burrows has capably discussed the phenomena of leukocytosis in response to theelin, and accompanying metaplasia, in two articles (16, 17). While he is the only worker to report the production of pyometra with theelin, Pierson and Morse, to judge from their pictures and descriptions, produced leukocytic infiltration and necrosis of the mucosal surface.
In the last months of our experiment the uterine horns of the mice receiving theelin were symmetrically thick, pale, and fibrous. We interpret this as a sequel to a preceding necrosis and leukocytic infiltration. Early turgidity and subsequent fibrosis seem a simple and adequate explanation for the reduced motility of the uterus (21).

Estrus: The estrous cycle was followed because of the report of Dodds (18) on the estrogenic action of the two carcinogenic hydrocarbons, 1:2-benzpyrene and 5:6-cyclol-2-benzanthracene. 1:2:5:6-Dibenzanthracene is not estrogenic by the usual test. The estrous cycle has not been followed in the course of tumor development with either tar or 1:2:5:6-dibenzanthracene.
Vaginal smears were taken twice a week on all of the animals throughout the experiment. This was, of course, too infrequent for precision, though it was probably sufficient to indicate any significant and consistent variation. The smears were taken in the manner described by Frank. A drop of water was washed in and out of the vagina with a glass pipette, which was inserted only into the introitus. This method was adopted as assuring the least mechanical effect on the vagina. The animals receiving theelin were all in
estrus three days later, when the next smear was taken. Sixteen weeks after the beginning of treatment all of the 31 spayed mice in Group III, which received only 1:2:5:6-dibenzanthracene, were in estrus. Previously they had been consistently in interval. Unfortunately at this time all treatment was discontinued for a week because of the mortality from benzene. At the next examination, 88 per cent were in estrus. They then returned to the interval stage until fifteen weeks later, when for a month the number in estrus fluctuated from 84 to 20 per cent. At the same time the incidence of estrus rose in Group I, normal animals receiving only 1:2:5:6-dibenzanthracene. Group I had shown no increase in the percentage of estrus at the time that all of Group III went into estrus. We have no explanation for this phenomenon; it is merely an observed fact. The increase in estrus in both in-

**Fig. 21.** 1. **Normal Uterus**; 2. **Hyperplasia from Theelin**; 3. **Carcinoma of Uterus**

Figs. 28 and 24 are from this carcinoma.

stances immediately preceded the appearance of the primary and secondary papillomas. If we lower the standard for reading estrus and call a smear with many epithelial cells and some leukocytes positive during the incidence of pyometra, then the animals receiving theelin remained in estrus throughout the rest of the experiment. No difference was noted in the estrous cycle of the animals that developed tumors and those that did not.

**Connective-tissue Tumors:** The connective-tissue tumors were not numerous, and were varied in type (Fig. 33). There were 9 in all. They occurred in all of the groups and all were located about the neck or mediastinum. This suggests that the chemicals penetrated the skin and acted on the underlying stroma, or by lymphatic drainage reached the mediastinum. That a nipple in one case and a breast in another was seen in the microscopic sections of the fibromas raises the question of some of these lesions being primary in the breast. The connective tissue of the genital tract in the animals treated with theelin appeared myxomatous. No fibromyomas of the uterus were found. The small number of connective-tissue tumors and their diversity of type permit no generalization in reference to the groups used in this experiment.
DEVELOPMENT OF TUMORS IN FEMALE MICE

1:2:5:6-Dibenzanthracene: Animals treated with only 1:2:5:6-dibenzanthracene developed carcinomas of the skin, lung, breast, stomach, and colon. These are the first carcinomas of the breast and alimentary tract to be produced with 1:2:5:6-dibenzanthracene. Hyperplasias and papillomas occurred in the viscera. Myofibromas, lymphoblastoma, and thymoma were also produced. The diverse types of tumors produced by 1:2:5:6-dibenzanthracene in this experiment are in accord with previous reports of its non-specific action.

1:2:5:6-Dibenzanthracene and Theelin: As we were not able to treat groups of mice with theelin alone, we cannot evaluate the proportionate action of the two chemicals. More tumors, and more varieties of tumor developed, and developed earlier, in the groups receiving both substances.

![Fig. 22. Uterus: Hyperplasia](image)

There are only two reports in the literature of the use of multiple chemical factors in producing carcinoma. Pierson's production of carcinoma of the uterus in rabbits with tar and folliculin has been discussed. Schockaert (19) reported that the mice treated with both tar and folliculin developed more carcinomas of the skin earlier and they grew more rapidly than the controls. He made weekly injections of 20 I.U. of theelin in oil.

Theelin: Gardner, Smith, Strong and Allen (20) produced a few sarcomas in mice with estrin. Cramer (6) has reported that in mice painted with 0.01 per cent of estrin in chloroform (1/10 of the concentration we used) twice a week, the males of a spontaneous mammary carcinoma strain developed carcinoma of the breast. In 8 of 12 mice treated with estrin for a long time, the pituitary was enlarged, and 3 had pituitary adenomas. Cramer also noted cachexia, atrophy of the spleen, testes and thymus, degeneration of the adrenals, and hypertrophy of the islets of Langerhans. We found in our
FIG. 23. UTERUS: METAPLASIA

FIG. 24. CROSS-SECTION OF UTERUS AT LEVEL OF CERVIX
A. and B. Cervices. C. and D. Ureters. E. Bladder

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Fig. 25. Cervix Uteri: Polyp with Hyperplasia, Cross-section

Fig. 26. Cervix Uteri: Carcinoma, Cross-section
FIG. 27. CERVIX UTERI: LONGITUDINAL SECTION, HYPERPLASIA, CYSTS, AND ATYPICAL EPITHELIUM

FIG. 28. SAME TUMOR AS FIG. 24, HIGHER MAGNIFICATION
late cases marked atrophy of the spleen, degeneration of the adrenals, and cachexia; we did not examine the pituitaries. Cramer (6) concludes: "Unlike any other carcinogenic substances so far studied experimentally, the carcinogenic effect of estrin is restricted to a tissue remote from the site of application of the carcinogenic agent but possessing a specific physiological sensitiveness to it. The action of estrin resembles that of the other carcinogenic agents in reproducing first a hyperplasia of the tissues in which the cancer subsequently develops—the precancerous condition—and in the long period of time necessary to induce cancer."

The only physiological studies made were those of vaginal smears. It was observed that the last mouse in Group IV persistently built nests for about a month before death. The last mouse in Group II showed a less persistent

Fig. 29. CERVIX UTERI: CARCINOMA, LONGITUDINAL SECTION

tendency to nest building. Both these mice had carcinomas of the uterus. All of one group of mice were caged together.

Non-neoplastic Changes: At about the 4th month, the animals receiving theelin showed marked enlargement of the clitoris and a gaping nodular vaginal orifice. These enlargements receded in the latter part of the experiment. Sections of the vaginal orifice and perineal region showed several changes which would produce the observed enlargement. The para-urethral glands (Fig. 32) and pars spongiosa of the clitoris were markedly hypertrophied. The epithelium was frequently atypical and metaplastic. Cysts developed in the glands. The mammae and anal glands were also hyperplastic.

Besides neoplasms, a number of other lesions developed. Abscesses of the skin and uterus have been mentioned. Amyloid was deposited in the viscera, necrosis of the liver and kidney occurred with acute benzene poison-
Fig. 30. Cervix Uteri: Longitudinal Section, Showing Hyperplasia of Squamous Epithelium Extending into Uterus

Fig. 31. Vaginal Polyp
FIG. 32. Paraurethral glands (A and B) which are hyperplastic and cystic. C. Clitoris. D. Urethra

FIG. 33. Myofibroma
ing, and subsequently fibrosis. There were several cases of ascites, and one of marked chylous ascites. Some of the cases of ascites were due to greatly enlarged mesentric lymph nodes rather than to cirrhosis of the liver.

CONCLUSION

A report is made of the production of neoplasms in a colony of female mice, half of which were treated with 1:2:5:6-dibenzanthracene and the other half with 1:2:5:6-dibenzanthracene and theelin. Numerous benign epithelial proliferations of the skin, breast, uterus, alimentary tract, and lungs occurred before the development of carcinoma and appear to be causally related to the subsequent malignancies. The incidence of carcinoma of the skin is chronologically related to the development of carcinomas of the breast. Carcinomas of the breast are causally and chronologically related to carcinomas of the uterus.

NOTE: It is a pleasure to express our sincere thanks to Professor C. L. Connor for the opportunity to make this study, and to thank Miss Pearl Hall and Mr. William Hewitt for the preparation of the microscopic sections.

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