RAPID INDUCTION OF MAMMARY CARCINOMA IN THE RAT AND THE INFLUENCE OF HORMONES ON THE TUMORS*

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It has been known for some years that certain aromatic hydrocarbons induce mammary tumors in some species of rodents. This paper is concerned with the conditions required for the rapid and invariable induction of mammary cancer in the rat by administering 3-methylcholanthrene *via* the gastro-intestinal tract and with some of the functional characteristics of the tumors which were evoked. During the experiments, a novel observation was made when it was found that the majority of the induced carcinomas of the breast are hormone-dependent, since they regress when the supporting internal secretions are removed. Whilst the concept of hormone dependence has been established in human mammary cancer, this property has not been recognized previously in mammary carcinomas of laboratory animals.

Tumors develop preferentially in the mammary gland of certain strains of mice and rats following the remote administration of some aromatic compounds; the local application of such carcinogens to the region of the mammary gland is unnecessary for the production of breast tumors. This effect was discovered by Lacassagne (1) who found that the administration of estrone induced mammary cancer in mice of the R III strain.

But mammary cancer can be evoked by compounds without hormonal activity; amongst these carcinogens are 3-methylcholanthrene (2), 2-acetylaminofluorene (2-AAF)[1] (3, 4), and 7,12-dimethylbenz-a-anthracene (DMBA)[2] (5). This remarkable activity of non-estrogenic carcinogens in inducing cancers of the mammary glands was first observed by Maisin and Coolen (2) who painted the skin of mice with 3-methylcholanthrene and found that, in addition to production of skin cancer, carcinoma of the mammary gland developed in high incidence. Mammary tumors have also been elicited with great frequency after the repeated instillation of 3-methylcholanthrene in the mouth (6), nares (7), or stomach (8) of the mouse or rat. Repeated intravenous injections of DMBA evoked similar tumors (5).

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[1] 2-AAF, 2-acetylaminofluorene.

[2] DMBA, 7,12,-dimethylbenz-α-anthracene.

The hormonal status of the treated animals, their lineage, and the dosage of the carcinogen are factors which have been identified as significant in the induction of mammary cancer by aromatic compounds. Englebreth-Holm (9) observed that painting the skin of mice with 3-methylcholanthrene elicited mammary cancer only in the females. Mammary tumors do occur in males following the remote administration of the designated carcinogens but the incidence of tumors is less than in females and the latent period for tumor development is prolonged. Bielschowsky (4, 10) found that the presence of ovaries was of high importance in accelerating the development of mammary cancer in rats fed a ration containing 2-AAF since ovariectomy decreased the incidence of mammary tumors; a similar inhibiting effect of ovariectomy has been observed in rats to which 3-methylcholanthrene was administered (8). Cantarow *et al.* (11) observed that the incidence of mammary tumors in rats fed a diet containing 2-AAF was greatly enhanced by simultaneous treatment with progesterone. There is a lack of agreement concerning the influence of estradiol-17β on the induction of mammary tumors in rats treated concomitantly with the polycyclic hydrocarbons. One group of workers (5) reported that estradiol-17β accelerated the appearance of the mammary cancers and increased their incidence, whilst in other experiments (12) this estrogen did not modify the effect of the mammary carcinogens. Shay *et al.* (13) found that estradiol-17β, and testosterone as well, lessened the incidence of mammary cancer in methylcholanthrene-treated rats.

Bielschowsky (14) found that the administration of 2-AAF in the food evoked mammary cancer in 70 per cent of Wistar rats but in only 4 per cent of piebald rats. The daily feeding of 4 mg. of 2-AAF was highly effective (10) in producing mammary cancer in rats but lowering the daily dosage to 1 mg. caused a considerable delay in carcinogenesis and reduced the number of tumors. Similarly, Geyer *et al.* (5) found that the level of dosage of DMBA was of importance in tumor production. Following intragastric instillation, 3-methylcholanthrene was detected in the milk of lactating rats (15).

Most of the mammary tumors evoked by the carcinogens in rats have been classified as adenocarcinoma (10). Sarcomas of the mammary gland (16) and benign tumors (fibroadenoma; adenoma) (10, 16) occur much less commonly. The carcinoma infiltrates the adjacent muscles and metastasis (8, 10) has been observed although it is not frequent. Bielschowsky (4) succeeded in transplanting the induced mammary cancers to other rats and transplantation has been done successfully by others (12, 16) but "takes" are rare.

In mice the single subcutaneous implantation of 3-methylcholanthrene, 1 mg., was followed by the development of mammary carcinomas in 84 to 525 days (17). In the experiments of Shay *et al.* (8) the intragastric administration of 3-methylcholanthrene, 2 mg. daily, induced mammary tumors in all intact female rats; the time of appearance of the tumors was from 129 to 383 days. Scholler and Carnes (12) injected DMBA intravenously in rats and achieved the most rapid induction of mammary cancer which has been reported hitherto; the median time of appearance of mammary tumors was 59 days and 89 *per cent* of these rats developed cancer of the breast in 14 weeks.

Bielschowsky (10) found that established mammary carcinomas, induced by carcinogens in the rat, did not diminish in size after ovariectomy; but these neoplasms frequently regressed, sometimes profoundly, during lactation to reappear later—an

observation similar to that made by Haddow (18) and Foulds (19) in certain spontaneous mammary tumors of mice.

Methods

The experiments were carried out under standardized conditions. Albino rats of the Sprague-Dawley strain were obtained from the dealer at age 42 days and kept thereafter in a controlled climate. All of the rats seemed to be free from infectious disease during the experiment. Most of the rats were fed a commercial ration; hypophysectomized animals were maintained on a synthetic, high protein (18 *per cent*) diet (20) consumed *ad libitum*. All operations were carried out under ether anesthesia. Each of the experiments reported in this paper was repeated at least 3 times and in all 1860 rats were studied.

Aromatic hydrocarbons were administered by gastric intubation, always beginning at age 50 days; at this time the rats weighed approximately 140 to 150 gm. The compounds were dissolved in sesame oil and were heated for 1 hour at 100°C. to promote their solubility and to sterilize them. *Throughout this paper dosage refers to the amount administered each day.* The stomach tube was a soft rubber (No. 10 French) catheter 6 cm. long which was attached to the hub of a hypodermic needle; a plastic tube 1 cm. in length was slipped over the upper part of the catheter. The rats were examined at frequent intervals for the appearance of palpable mammary tumors. The growth of many of the established tumors was followed by photography at frequent intervals and by measurement of the greatest diameters from which the "surface area" was calculated.

At necropsy the tumors and the inguinal mammary glands were immersed in chilled acetone or neutral formalin. Histologic preparations were stained with hematoxylin and eosin, with Schiff's reagents (PAS) and with mucicarmine. The site of alkaline phosphatase was determined in slices of the tumor and the mammary glands by the method of Gomori (21). The content of alkaline phosphatase was determined by the method of King and Armstrong (22) on homogenates.

EXPERIMENTAL

Mammary tumors were always the first neoplasms which were detected in carcinogen-fed rats in the present experiments and often they were the only tumors to be observed. The mammary cancers manifested themselves first as indurated areas in the skin or as small shot-like nodules in the deeper tissues; in untreated intact females both types of lesions increased in size in a few weeks subsequent to their appearance. Spontaneous mammary tumors have never been observed in our colony earlier than age 500 days.

In a series of 682 rats, multiple mammary cancers developed in all intact female rats receiving 3-methylcholanthrene, 10 mg. daily by gastric instillation. The location of the tumors was distributed in percentage as follows: cervical region, 25; thoracic region, 41; abdomino-inguinal, 34.

Influence of Dosage of 3-Methylcholanthrene.—Single feeding: In this experiment the compound dissolved in sesame oil was given once by stomach tube to 9 rats at each dose level. A single dose of 20 mg. elicited mammary cancer in 2 rats at 74 and 167 days, respectively; a dose of 50 mg. evoked mammary cancer in 3 rats in 44 to 83 days; a dose of 75 mg. induced mammary cancer in 4 rats between 43 and 69 days.

Multiple feedings: In this experiment, 3-methylcholanthrene was administered by stomach tube 6 days each week. When 3-methylcholanthrene was administered at a dosage of 2 mg. mammary tumors developed slowly and many rats were free from tumors at 9 months (Table I). Doses of 5 mg., or 10 mg. elicited mammary cancer in all intact rats, the rate of their development being faster with a dosage level of 10 mg.

Rats receiving 3-methylcholanthrene, 10 mg. daily by gastric intubation tolerated the procedure well; in a series of 113 rats, 4 animals died before 100 days. Severe general toxicity was evident when the dosage of 3-methylcholanthrene was increased to 15 mg.; 66 per cent of the rats (Table I) died before mammary tumors developed. At a dosage of 20 mg., all rats died before 40 days.

TABLE I

Effect of Dosage of 3-Methylcholanthrene on the Incidence of Palpable Mammary Tumors and Time of Appearance

The compound was administered by stomach tube 6 days each week until all rats had developed tumors or the animals had been observed for 9 months.

Daily dosage	No. rats	Rats with tumors	Appearance of palpable tumors, *days*		
			Range	Median	Mean
mg.					
2	14	3	69–156	—	—
5	40	40	47–156	64	67.9
10	109	109	22–77	50	55.9
15	24*	24	34–69	49	49.2
20	15	0	All rats died between 24 and 40 days		

* In all 72 rats were treated at this dosage level; 48 died without tumors 34 to 69 days after beginning the experiment.

Influence of Various Schedules of Feeding 3-Methylcholanthrene.—3-Methylcholanthrene was given by stomach tube daily to groups of normal female rats for consecutive periods of 10 to 50 days; the incidence of palpable mammary tumors and the time of their appearance are given in Table II. In the group of rats fed 3-methylcholanthrene, 10 mg. for 10 days, not all of the rats developed mammary cancer in 5 months and the time of appearance of tumors was more than 4 months in some of the rats. The administration of 3-methylcholanthrene, 10 mg. for 20 to 50 days elicited mammary cancers with nearly the same rapidity in all of the groups (Table II).

In another experiment, groups of rats were given 3-methylcholanthrene, 10 mg., by stomach tube for 50 days with differing frequencies varying from 1 day to 6 days of each week; the days of administration of the compound remained constant. All of the rats developed mammary cancer, regardless of

feeding 1 day or 6 days each week. The development of mammary cancer was somewhat retarded in some of the rats receiving the compound at weekly or semiweekly intervals. Giving the compound thrice weekly (Mon., Wed., Fri.) was nearly as effective in the rapid induction of mammary cancer as more frequent administration (Table III).

Influence of Hormonal Status on the Development of Mammary Cancer.—In comparison with the findings in intact rats, the incidence of mammary cancers was reduced and the time of their appearance was retarded in a *group* of rats which had been ovariectomized at age 42 days and which had been fed 3-methylcholanthrene, 10 mg. (Table IV). But in this group mammary cancer was observed early (at 49 and 59 days, respectively) in 2 of the ovariectomized rats which, at necropsy were found to have the atrophy of the uterus and vagina which is characteristic of ovarian ablation.

TABLE II

Influence of Duration of Administration of 3-Methylcholanthrene on Development of Palpable Mammary Tumors

3-Methylcholanthrene, 10 mg., was administered by stomach tube for varying periods of consecutive days. There were 10 rats in each group.

Time of administration	Rats without tumors	Appearance of tumors, *days*		
		Range	Median	Mean
days				
10	3	48–142	59	78.9
20	0	32–66	55	51.5
30	0	41–66	51	54.1
40	0	41–58	48	52.1
50	0	48–76	55	56.9

The inhibitory effect of ovariectomy on the development of mammary cancer in rats fed 3-methylcholanthrene, 10 mg., was overcome by the daily injection of estradiol-17β, 0.1 or 1 μg.; such quantities induced estrus in all of the ovariectomized rats. Mammary cancer developed in all of the animals but not quite so quickly as in intact controls (Table IV). It was found that large quantities of estradiol-17β (20 μg. daily) depressed the incidence of mammary cancers and retarded the time of their appearance (Table IV).

In accord with earlier work with another carcinogen (11), the repeated intramuscular injection of progesterone, 4 mg. daily, enhanced the rapidity of formation of mammary cancer in rats fed 3-methylcholanthrene, 10 mg., concurrently. A novel and unexpected finding was the rapid induction of mammary cancer in all ovariectomized rats injected with progesterone, 4 mg. and fed 3-methylcholanthrene, 10 mg. (Table IV).

The daily injection of dihydrotestosterone 1 mg., delayed considerably but

did not prevent the formation of mammary cancer in rats fed concurrently with 7,12-dimethylbenz-a-anthracene, 1 mg. (Table V).

Physiological Effects of Oral Administration of 3-Methylcholanthrene.—3-Methylcholanthrene, 10 mg. in 1 cc. of sesame oil, was administered 6 days each week by gastric intubation to a group of 10 rats; their mates were fed an equal amount of sesame oil.

TABLE III

Influence of Frequency of Administration of 3-Methylcholanthrene on Induction of Palpable Mammary Tumors

3-Methylcholanthrene, 10 mg., was administered by stomach tube according to various schedules of administration for 50 days: 10 rats in each group.

Frequency of administration, *days each week*	Appearance of tumors, *days*		
	Range	Median	Mean
6	37–58	44	47.8
5	44–64	51	54.1
4	44–64	56	55.9
3	44–56	51	50.1
2	37–85	52	58.4
1	49–100	69	67.3

TABLE IV

Influence of Ovariectomy, Estradiol-17β, and Progesterone on Incidence of Palpable Mammary Tumors in Rats Treated with 3-Methylcholanthrene

3-Methylcholanthrene, 10 mg., was given to all of these female rats by stomach tube, 6 days each week for 50 days. Steroids were injected intramuscularly. The rats were observed for 120 days.

Category	Dosage of steroid	No. rats	Rats with tumors	Appearance of palpable tumors, *days*		
				Range	Median	Mean
	Controls					
Intact	0	10	10	37–63	51	50.6
Ovariectomized	0	10	6	49–118	78	73.7
	Estradiol-17β					
Ovariectomized	0.1 μg.	9	9	41–88	55	63.1
Ovariectomized	1	9	9	55–83	72	72.1
Ovariectomized	20	9	3	80–97	85	—
	Progesterone					
Intact	4 mg.	10	10	30–51	41	40.3
Ovariectomized	4 "	10	10	48–74	56	58.3

All of the carcinogen-fed rats had developed mammary cancer after 50 days of treatment (Table VI). There was no difference from the control group in

total body weight but there was a considerable decrease in the weight of the pituitary, the ovaries, and the uterus (Table VI). The weight of the inguinal mammary glands, exclusive of those containing visible carcinoma, was similar

TABLE V

Influence of Dihydrotestosterone on Incidence of Palpable Mammary Tumors in Rats Treated with 7,12-Dimethylbenz-a-anthracene

7,12-Dimethylbenz-*a*-anthracene, 1 mg., was given by stomach tube 6 days each week for 100 days, to intact female rats. Dihydrotestosterone, 1 mg., was injected intramuscularly for 84 days; the controls received injections of sesame oil.

Category	No. of rats	Rats with tumors	Appearance of palpable tumors, *days*		
			Range	Median	Mean
Controls	9	9	47–100	79	78.9
Injected with dihydrotestosterone	9	5	161–204	168	177.2

TABLE VI

Physiological Effects of 3-Methylcholanthrene

3-Methylcholanthrene, 10 mg., was administered to intact female rats, age 50 days, by stomach tube, 6 days each week, for 50 days. Sesame oil was administered to the control rats. There were 10 rats in each group. Mean values are given; they were obtained at necropsy at age 101 days.

	Control	3-Methylcholanthrene-treated
Rats with mammary cancer	0	10
Body weight	225 gm.	226 gm.
Mammary gland weight*	352 mg.	365 mg.
Alkaline phosphatase content of mammary gland‡	0.79 ± 0.13 units	0.53 ± 0.08 units
Ovaries	72 mg.	52 mg.
Uterus	407 "	310 "
Adrenals	68 "	62 "
Pituitary	11.2 ± 1.4 "	8.7 ± 0.8 "

±, standard deviation.

* Inguinal mammary gland.

‡ Alkaline phosphatase expressed in King-Armstrong units (22).

in the two groups but the content of alkaline phosphatase was considerably lower in the mammary glands of the carcinogen-fed rats. A consequence of ovariectomy in the rat is a decreased content of alkaline phosphatase in the mammary glands accompanied by atrophy of epithelium (23). In the carcinogen-treated rats alkaline phosphatase content of the breast was decreased but

the mammary epithelium was not atrophic; to the contrary, a moderately increased epithelial proliferation was present (Fig. 1).

Pathology of Carcinogen-Induced Mammary Tumors. Histologic examination was made of 680 mammary tumors induced in intact female rats by the oral administration of 3-methylcholanthrene. These were classified in the following way: carcinoma, 678; fibrosarcoma, 2; benign tumors, 0. The fibrosarcomas in the mammary region developed in ovariectomized rats fed 3-methylcholanthrene, 5 mg., for 201 and 218 days, respectively.

The carcinomas in intact rats were white and soft. Hemorrhage and necrosis were frequently encountered in parts of the tumors but many were free from these complications; complete necrosis of a tumor was never observed. The growth showed a considerable similarity of cytologic pattern. The tumors contained acini lined with many layers of epithelial cells (Fig. 2) arranged to form gland-like structures with papillary projections in the lumina. The lumina of these glandular structures were filled with eosinophilic material containing protein and, apparently, carbohydrate since it stained deeply with mucicarmine and the PAS reagents; this secretion also contained considerable amounts of alkaline phosphatase. The alkaline phosphatase reaction also served to identify myo-epithelial cells which were present in great numbers irregularly organized around and within the neoplastic glands (Figs. 2, 3).

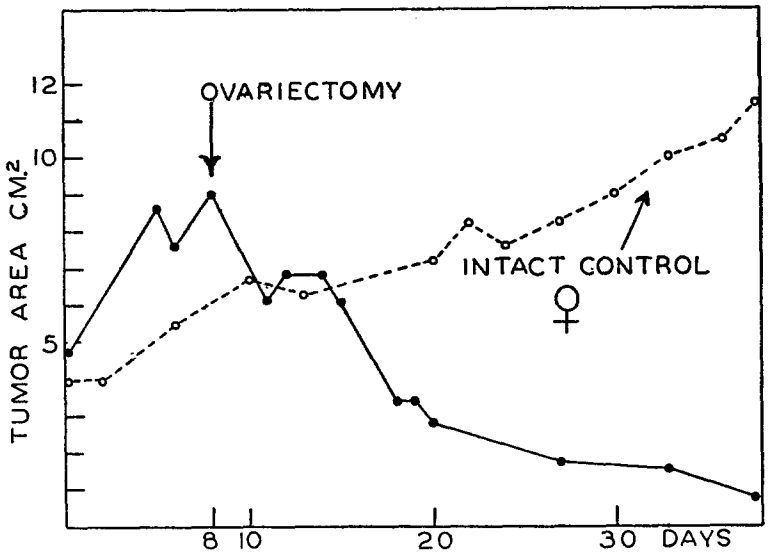
The earliest neoplastic changes in the mammary glands of intact rats were observed 20 days after beginning the feeding of 3-methylcholanthrene; at this time, a considerable proliferation of epithelial cells (Fig. 4) was observed in areas of the mammary gland with penetration of the neoplasm through the normal acinar confines; other mammary glands of this rat did not differ cytologically from those of untreated normal females of the same age. It was observed that the neoplastic process arose in both acini (Fig. 5) and smaller ducts (Fig. 6) of the breast. The nipples and terminal milk ducts never were found to have developed neoplasms.

The tumors induced with 3-methylcholanthrene in ovariectomized rats differed morphologically from the neoplasms elicited in intact rats. The chief difference was in the epithelial cells and in secretion of the tumor acini in the spayed rats. Whereas in both intact and ovariectomized rats there occurred neoplastic gland-like structures lined with many layers of cells and surrounded by myo-epithelial cells, the secretion in the lumina of the tumor acini of ovariectomized animals was devoid of alkaline phosphatase and carbohydrate. In the mammary tumors of spayed rats, some of the acini were lined with a single row of flat cells (Fig. 11), whilst other glands consisted of many layers of epithelial cells. Acini of these types were frequently observed in mammary cancers following the removal of the ovaries of intact rats, a matter to be considered later in this paper.

Distant metastases of the induced mammary cancers were not observed, but

infiltration of adjacent muscles, skin, and the mammary fat were observed frequently. Large tumors frequently developed ulcers which caused the death of the animal.

Hormone Dependence of Induced Mammary Cancer.—Many of the established mammary cancers induced by 3-methylcholanthrene were hormone-dependent. These underwent a considerable diminution in size after appropriate hormonal modifications of the host and the regression was accompanied by atropic changes, which were often profound, in the tumor cells. Less frequently, these



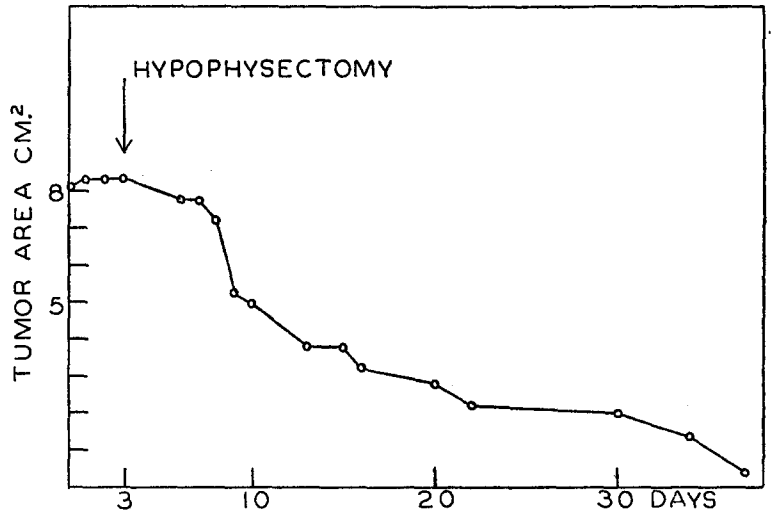
TEXT-FIG. 1. Decline in surface area of an established mammary carcinoma induced by ovariectomy (●——●) is contrasted with continued growth of the neoplasm in its untreated sister (O- -O). Ovariectomy was performed on day 8.

same hormonal treatments failed to cause regression of the carcinoma and the characteristic atrophy was absent.

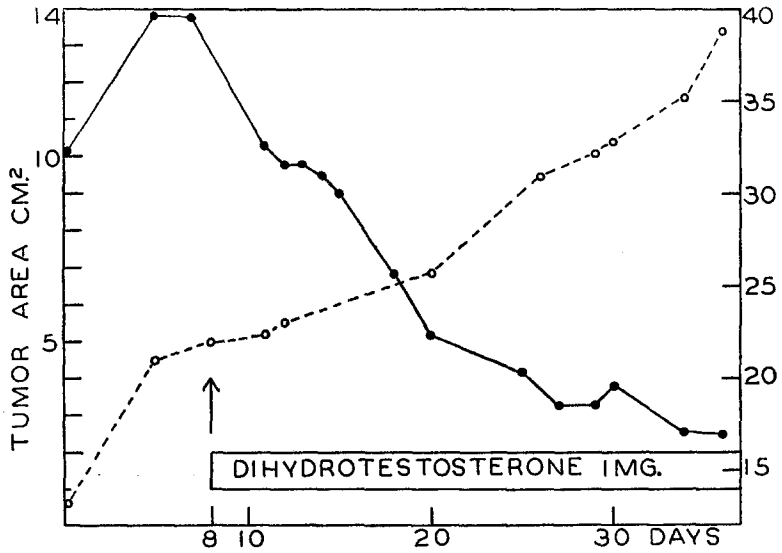
In one of the experiments, tumors were induced by giving 3-methylcholanthrene, 10 mg. daily, by stomach tube for 50 days, at which time 54 rats had developed mammary cancer; the administration of the carcinogen was then discontinued. After an additional 25 days, the rats were divided into groups which were, respectively, (*a*) untreated controls, (*b*) injected with dihydrotestosterone, (*c*) ovariectomized, or (*d*) hypophysectomized. Another group of ovariectomized rats was injected intramuscularly with diethylstilbestrol, 5 μg.

The tumors increased in size in all of the intact rats (Text-fig. 1) and also in ovariectomized rats injected with diethylstilbestrol (Table VII).

All of the tumors underwent a profound decrease in size (Text-fig. 2) after



TEXT-FIG. 2. Profound decrease in surface area of an established mammary carcinoma following hypophysectomy on day 3.



TEXT-FIG. 3. Divergent growth response of mammary carcinomas of 2 rats to the daily injection of dihydrotestosterone. The surface area of the tumor of 1 of the rats (●——●) decreased profoundly; the neoplasm in another rat (O– –O) grew at a rapid rate.

hypophysectomy. In 2 rats, small masses of mammary cancer (approximately 0.5 x 0.5 cm.) were present 50 days after hypophysectomy; in 7 rats only tiny fibrotic masses were found.

Ovariectomy was performed on 8 rats bearing carcinogen-induced cancer of the breast. There was no uniformity in the growth rate of the mammary cancers after this procedure. Indeed, in 7 rats the tumor underwent a considerable decrease in its size (Table VII) while in one animal the tumor continued to grow despite deprival of the ovaries. There was a similar divergent effect of growth of tumors in rats injected daily with dihydrotestosterone, 1 mg. or 2 mg. This

TABLE VII

Influence of Hormonal Modifications on Growth of Established Mammary Tumors in Female Rats

Mammary cancer was induced in 54 rats by the intragastric administration of 3-methylcholanthrene, 10 mg., for 50 days; the compound was then discontinued. 25 days later, the rats were subjected to hormonal modifications and the tumors were measured for an additional 50 days.

Category	No. rats	Increased size of tumor	Decreased size of tumor
Intact: no treatment	10	10	0
Intact + dihydrotestosterone, 1 mg.*	9	2	7
Intact + dihydrotestosterone, 2 mg.*	8	1	7
Ovariectomized	8	1	7
Ovariectomized + diethylstilbestrol, 5 μg.*	10	10	0
Hypophysectomized	9	0	9

* Dihydrotestosterone and diethylstilbestrol injected intramuscularly.

steroid was administered to 17 intact tumor-bearing animals. A considerable regression of mass of the mammary cancer occurred in 14 animals (Figs. 7, 8) whilst the tumor size increased greatly in 3 rats (Fig. 9, 10). The two types of response, respectively, continued growth or profound depression, are represented quantitatively in Text-fig. 3.

The mammary cancers which diminished in size after the endocrine modifications (ovariectomy, hypophysectomy, administration of dihydrotestosterone) exhibited thereafter a characteristic cytologic appearance of atrophy of epithelial cells, which was similar in all groups. The tumor acini were still surrounded by myo-epithelial cells but their many layers of plump epithelial cells were replaced by a single row of flat cells (Figs. 12, 13); moreover, the acinar contents no longer contained cytologically detected amounts of alkaline phosphatase or carbohydrate. There was a massive increase in the interacinar interstices of cells rich in alkaline phosphatase.

The tumors which did not decrease in size after the hormonal modifications contained acini lined with many layers of epithelial cells (Fig. 14). In the acini

of such growths, the secretion characteristic of the tumors of intact rats was absent.

In the tumors of 2 animals there was evidence that some of the cells had undergone hormone-withdrawal atrophy in response to ovariectomy whilst others were hormone-independent in character. In some areas of the tumor there were atrophic acini (Fig. 15) lined with a single layer of epithelial cells, and there was an abundance of cells rich in alkaline phosphatase between the neoplastic alveoli (Fig. 16). In adjacent areas of the tumor, acini lined with many layers of cells were seen without the dense concentration of alkaline phosphatase (Fig. 15, 16). Clearly, the cell population of these tumors was not uniform in its functional or morphologic response to ovariectomy; since hormone-independent cells were abundant, the tumor continued to grow despite the presence of a hormone-dependent component.

DISCUSSION

Under optimal circumstances 3-methylcholanthrene administered by way of the gastrointestinal tract has a special and, indeed, an overwhelming activity in producing mammary cancers in the rat and with unprecedented speed. Conditions were found in the present study rendering it possible to evoke multiple cancers of the breast in every animal in less than 2 months; no exceptions occurred in 18 consecutive series of experiments on a total of 180 animals. Mammary cancer was always the first neoplasm which was detected and usually was the only tumor found but the rats were not kept until they became very old.

The earliest carcinoma was detected 20 days after beginning the feeding of 3-methylcholanthrene and many cancers appeared before 30 days. If there were a latent period of carcinogenesis, it must have been brief indeed in the case of these tumors. These rapid effects may be compared with those of other accelerated methods of cancer induction. Bachmann and the Kennaways (24) applied 5,9,10-trimethylbenz-a-anthracene to the skin of mice and observed a cutaneous papilloma on the 31st day. Rous (25) injected a cell-free filtrate of the Rous Chicken Sarcoma I in other fowls and observed the first palpable tumors 10 to 21 days thereafter.

To be most serviceable as a laboratory procedure for the study of cancer of the breast, a method of induction must be able to induce mammary cancer in all of the animals in a short time. These conditions were fulfilled in the present experiments. According to our concept, mammary cancer can be considered to have been induced rapidly whenever every animal in an experimental group develops this tumor within 60 days. Under the conditions employed in the present study, the rapid induction of mammary cancer through the administration of 3-methylcholanthrene is a function of (*a*) dosage of the compound, (*b*) frequency of its administration, and (*c*) favorable hormonal conditions in the recipients. The dosage of the compound must not be excessive; a daily amount

of 3-methylcholanthrene, 15 mg. (or larger amounts) given for a long time killed many rats before mammary cancer arose.

Mammary carcinoma was induced in some rats by a single dose (20 to 75 mg.) of 3-methylcholanthrene but this result was inconstant since other rats treated similarly did not develop cancer. For the rapid development of mammary cancer in the present work the optimal dosage of 3-methylcholanthrene was 10 mg. administered 6 times each week by stomach tube for 7 weeks.

The importance of the schedule of administration of the mammary carcinogen was brought out in an experiment in which small amounts of 3-methylcholanthrene were administered to 2 groups of rats. One group received 3-methylcholanthrene, 10 mg., at daily intervals for 10 days (total dosage 100 mg.) and not all of these animals had developed mammary cancer after 5 months. But when 3-methylcholanthrene, 10 mg., was administered at weekly intervals for 7 weeks (total dosage 80 mg.) every rat developed mammary cancer within 92 days.

The hormonal status of intact female rats, age 50 days, proved conducive to the induction of mammary cancer by 3-methylcholanthrene but it was not optimal for the development of these neoplasms. The repeated administration of 10 mg., of the carcinogen throughout prolonged periods caused little or no loss of weight but resulted in considerable changes in endocrine activity. In rats, so treated, there was a significant though *partial* depression of hypophyseal and ovarian functions; atrophy of the mammary epithelium did not occur but instead there was a mild hyperplasia of the mammary tubules and despite the decreased endocrine function mammary cancer developed rapidly. Similar hormonal effects and increased growth of the mammary gland had been observed earlier (26), following the repeated intramuscular injection of 3-methylcholanthrene (1 or 2 mg. daily); in those experiments mammary cancer did not occur, presumably because the dosage of the aromatic compound was insufficient for carcinogenesis.

Ovariectomy retarded the development of mammary cancer in many but not in all of the animals. When 3-methylcholanthrene, 10 mg., was fed daily to ovariectomized rats for prolonged periods, there was a significant slowing of the average time of development of the cancers although 2 members of a group of 10 spayed rats developed cancer of the breast before the 60th day.

It has been shown (23) that estradiol-17β exerts a biphasic effect on the growth of transplanted mammary fibroadenoma in spayed rats: small amounts of phenolic estrogens profoundly accelerated the growth of the transplants whilst large amounts blocked their growth. Once again, the biphasic (dosage: growth) effects of estradiol-17β on mammary tumors was seen. In the present experiments moderate amounts of estradiol-17β favored the incidence and growth of mammary cancer in ovariectomized rats which were fed 3-methylcholanthrene whilst large amounts of the hormone hindered their development.

Progesterone accelerated the induction of mammary cancer; the most rapid

development of cancer of the breast in rats fed 3-methylcholanthrene occurred in animals injected concurrently with large doses of this hormone. The tumor-accelerating action of progesterone was manifest not only in intact rats, but, unexpectedly, in their ovariectomized companions. Progesterone is known to enhance the growth of the mammary tree but only in rats in which mammary development has been incited by phenolic estrogens. In the present experiments with ovariectomized rats, progesterone, 4 mg., was very effective in accelerating the development of mammary cancers in animals fed 3-methylcholanthrene but in the absence of the aromatic compound, progesterone neither induced growth of the mammary epithelium nor evoked cancers. It is evident that 3-methylcholanthrene can substitute for phenolic estrogens in the primary incitement of mammary growth of the type that is promoted by progesterone.

The cytologic appearance of the carcinomas induced by 3-methylcholanthrene in intact rats was remarkably similar in all cases but divergent responses of the established tumors to hormonal modifications were observed. A uniform carcinogenic cause was not followed by tumors possessing identical physiologic characteristics; the elicited tumors varied in their functional response to hormonal changes in their hosts.

Many of the induced carcinomas, indeed the majority of them, regressed markedly in size when ovarian function was abolished by ovariectomy or by hypophysectomy; removal of the pituitary always was followed by the greatest decline in tumor size. Cancers behaving in this way are by definition *hormone-dependent.* This decrease in size was not due to necrosis but to atrophy of the epithelial cells, resulting in a characteristic histologic appearance.

Similarly, the administration of dihydrotestosterone caused hormone-dependent cancers to decrease in size; this compound, in common with other androgenic substances, is known to induce a decline of ovarian function yet, notably, promotes growth intensely in the normal mammary epithelium of the rat (27, 28). It was of interest to observe vigorous growth of the normal mammary tree in rats injected with dihydrotestosterone whilst the mammary cancers became atrophic in the same animals.

A minority of the induced mammary cancers continued to grow despite ovariectomy or the administration of dihydrotestosterone; tumors of this type are *hormone-independent.* In several cases, mammary tumors continued to grow after ovariectomy but, amidst the proliferating tissue, areas existed consisting of shrunken tumor cells (Figs. 15, 16). It would appear that not all of the cells even in a single tumor were uniform in their response to hormone withdrawal.

Some of the cells of the induced mammary cancers in the rat possess a biochemical mechanism that enables them to grow in the absence of those hormones which are essential for the growth of other cancer cells and of normal mammary epithelium as well.

In various strains of mice possessing the milk agent, spontaneous mammary cancers which have reached palpable size do not regress after ovariectomy, hypophysectomy, or the administration of testosterone (29). In this regard the mouse tumors differ from many mammary cancers of man. But the response of the carcinogen-induced mammary cancers of the rat to hormonal modifications of these kinds are reminiscent of the effects of these procedures on cancers of the human breast for which they serve as close experimental models which are unique at the present time.

CONCLUSION

A study was made of the optimal conditions for the induction of mammary cancer in the rat. 3-Methylcholanthrene was administered *via* the gastrointestinal tract, and a simple technique was worked out for inducing mammary cancer regularly and rapidly. Under conducive conditions, which were readily reproduced, multiple mammary carcinomas and these tumors only were induced in every rat in repeated experiments in 60 days or less. In the strain of animal employed in the present experiments, the rapid induction of mammary cancer proved to be a function of (*a*) dosage, (*b*) the timing of administration of the aromatic hydrocarbon, and (*c*) a favoring hormonal status of the recipient.

Most of the established tumors were *hormone-dependent* because they diminished markedly in size after hormone withdrawal through ovariectomy or hypophysectomy. Similar regression of the tumors was frequently achieved by the administration of dihydrotestosterone. Shrinkage of the cancers was accompanied by atrophic changes. Experimental mammary tumors with these physiologic characteristics have not been recognized hitherto.

The minority of mammary cancers continued to grow after ovariectomy; these are *hormone-independent* tumors and tumors of this sort had a characteristic cytologic appearance following modification of the endocrine state.

The cell population of a single tumor was not always uniform in its response to appropriate hormonal modifications. In certain tumors in response to changes in the endocrine status of the host many of the cells underwent atrophy whilst other adjacent cells in the same tumor continued to grow so that the net result was a hormone-independent tumor.

Hypophysectomy was the most effective method found to induce regression of mammary cancer in the present experiments.

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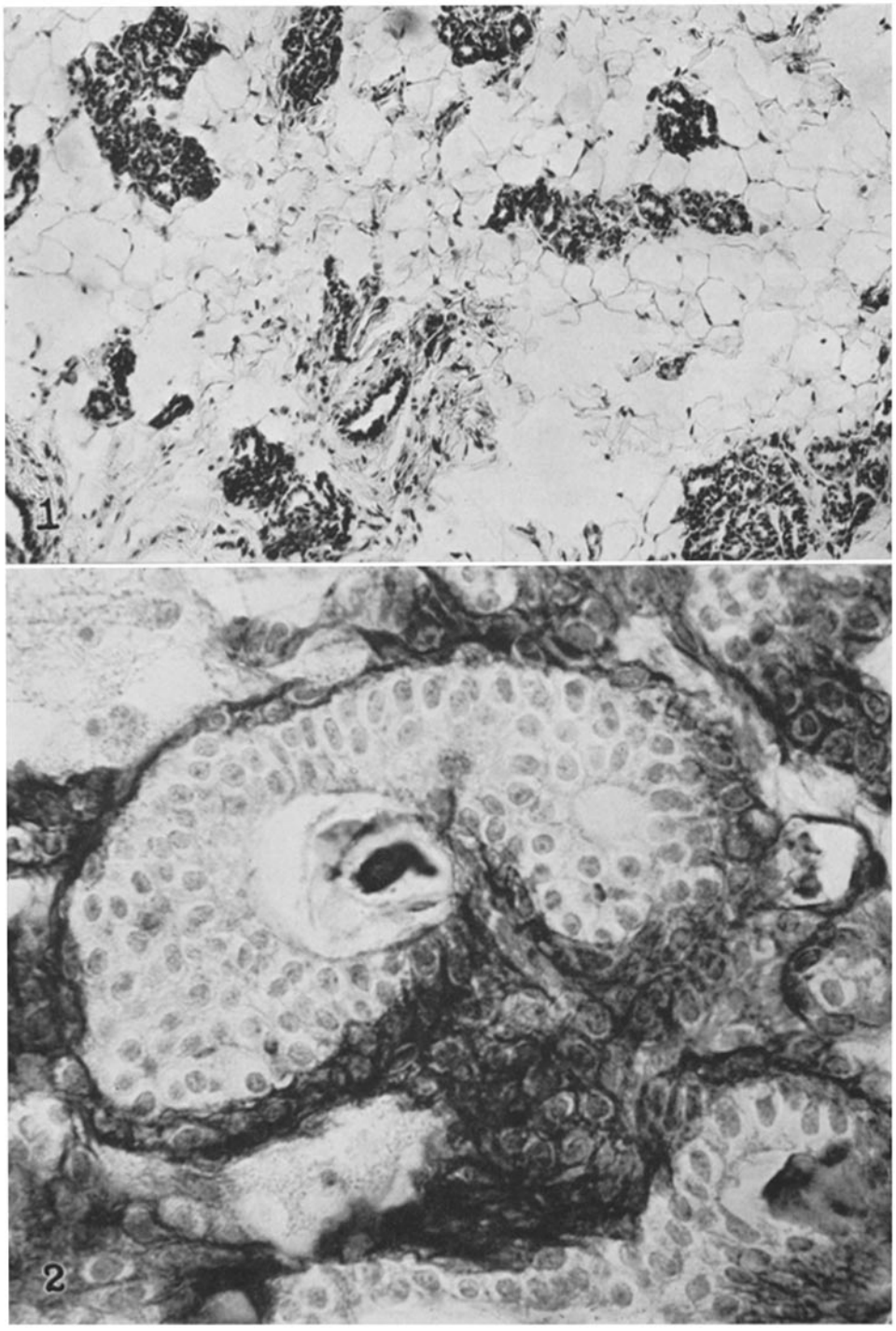
EXPLANATION OF PLATES

Unless stated otherwise, the photomicrographs are of paraffin sections stained with hematoxylin and eosin. In Figs. 2, 3, 5, and 16 the site of alkaline phosphatase was demonstrated by the method of Gomori (21).

PLATE 4

FIG. 1. Mammary gland of an intact female rat age 101 days showing slight hyperplasia of the tubules induced by the intragastric administration of 3-methylcholanthrene, 10 mg., for 50 days. × 150.

FIG. 2. The distribution of alkaline phosphatase in a mammary cancer induced by 3-methylcholanthrene in an intact female rat. The tumor acini, surrounded by myoepithelial cells, consist of many layers of epithelial cells; the secretion in the lumina contains alkaline phosphatase. × 730.

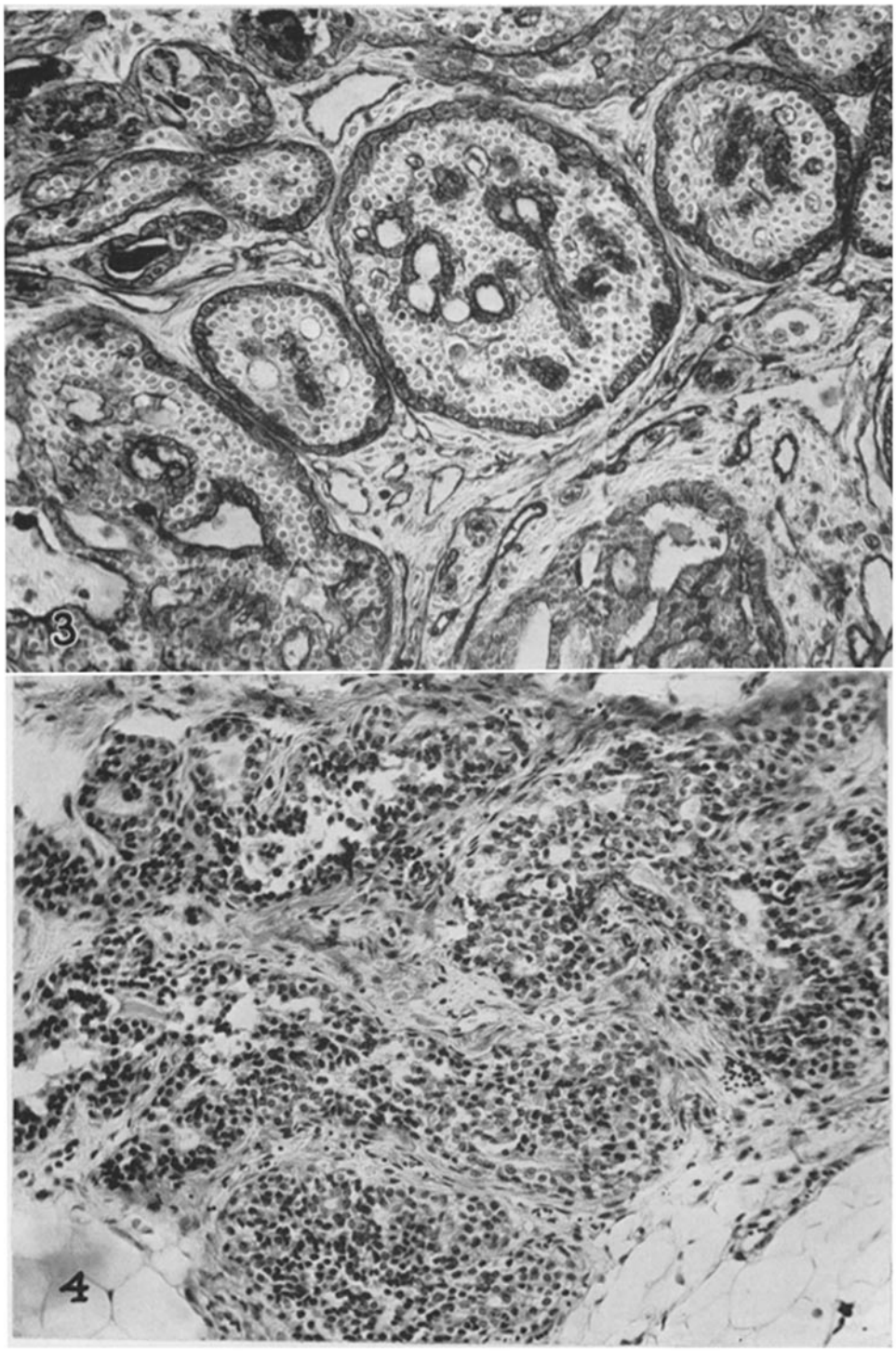


(Huggins *et al.*: Induction of mammary carcinoma)

PLATE 5

FIG. 3. The site of alkaline phosphatase in a mammary carcinoma arising in an intact female rat fed 3-methylcholanthrene. The enzyme is present predominantly in the capillaries, the acinar secretion, and in the myo-epithelial cells which surround the glands and are displaced amongst the epithelial cells. × 225.

FIG. 4. Mammary carcinoma which arose after feeding 3-methylcholanthrene, 10 mg., to an intact female rat for 20 days, at which time necropsy was performed. This tumor was the earliest one observed in the present experiments. × 250.

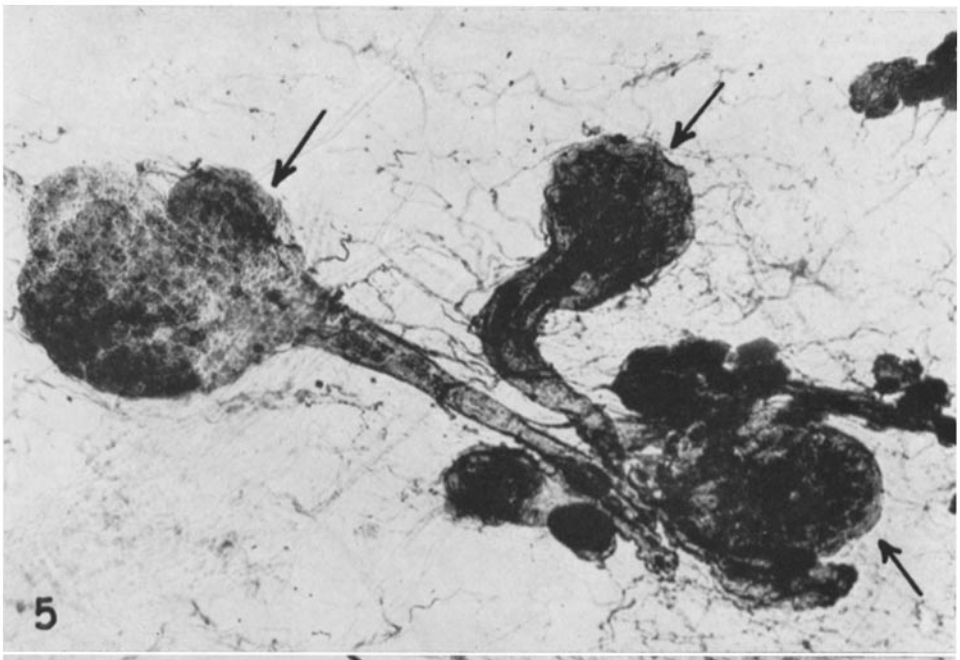


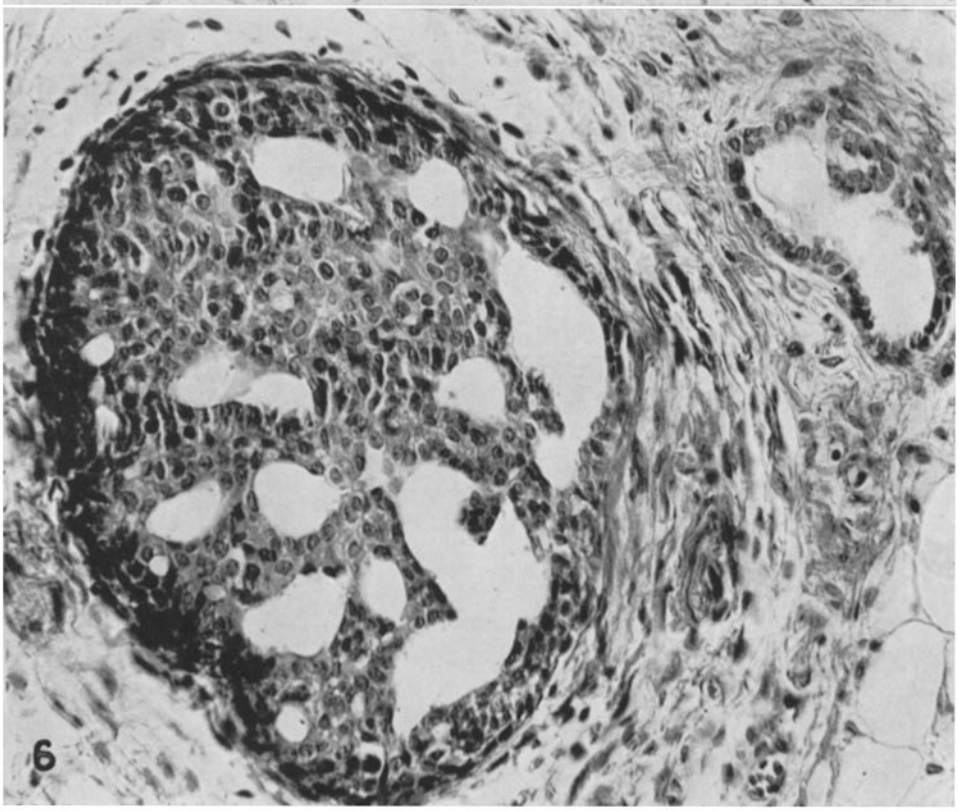
(Huggins *et al.*: Induction of mammary carcinoma)

PLATE 6

FIG. 5. Whole mount of the mammary gland of an intact female rat fed 3-methylcholanthrene for 50 days. The arrows point to carcinomas which have arisen in 3 of the acini; normal acini are shown on the right. × 65.

FIG. 6. Mammary carcinoma arising in a small duct in an intact female rat fed 3-methylcholanthrene for 30 days. A normal mammary duct is shown on the right. × 250.





(Huggins *et al.*: Induction of mammary carcinoma)

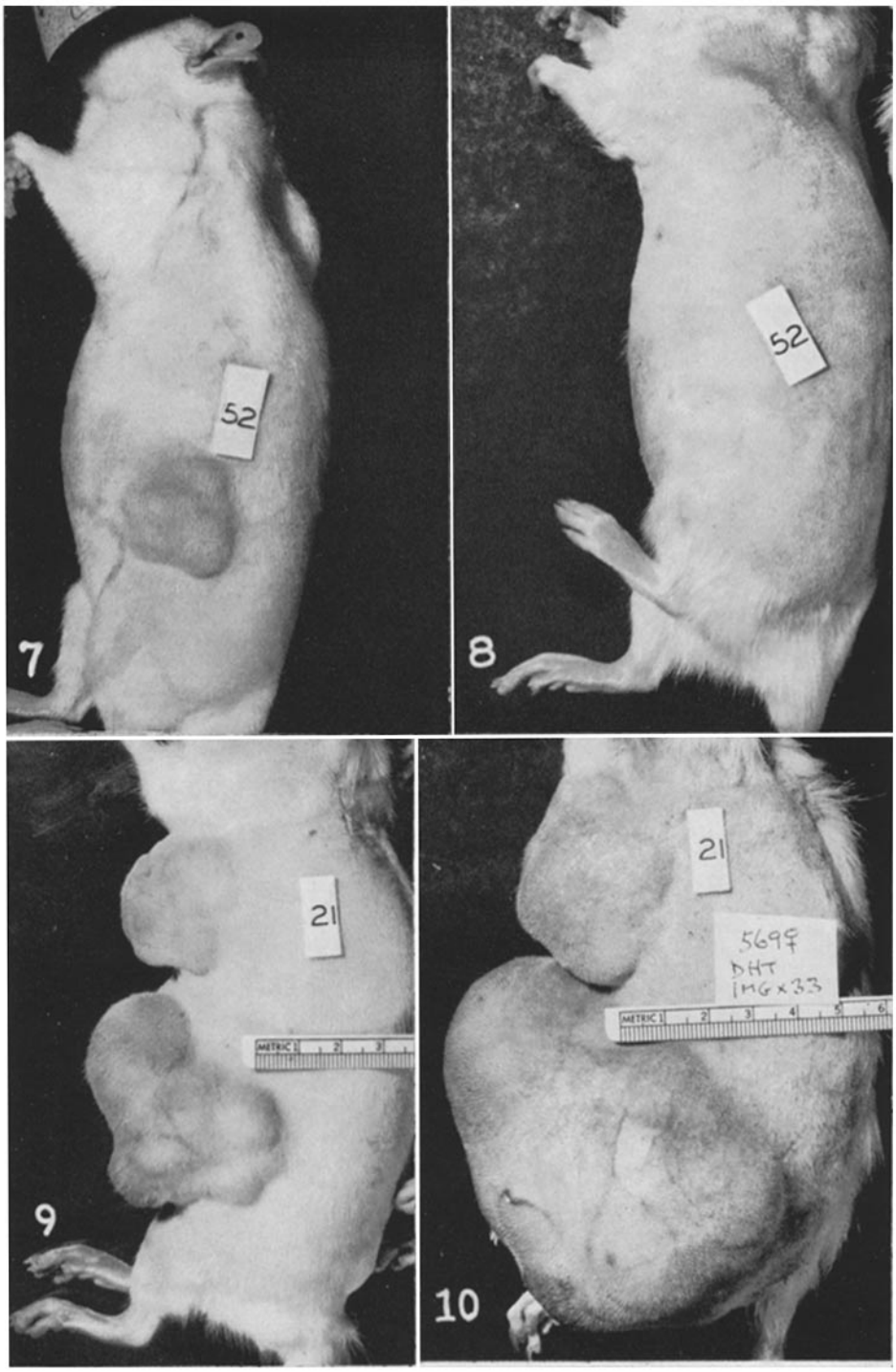
PLATE 7

FIG. 7. Multiple mammary carcinomas, induced in an intact female rat, by feeding 3-methylcholanthrene; this picture was taken before the administration of dihydrotestosterone.

FIG. 8. The same rat shown in Fig. 7. The mammary cancers have regressed following the injection of dihydrotestosterone, 1 mg., for 33 days.

FIG. 9. Mammary carcinomas, induced in an intact female rat, by feeding 3-methylcholanthrene, photographed before the administration of dihydrotestosterone.

FIG. 10. The same rat shown in Fig. 10. The mammary cancers have grown despite the injection of dihydrotestosterone, 1 mg., for 33 days.



(Huggins *et al.*: Induction of mammary carcinoma)

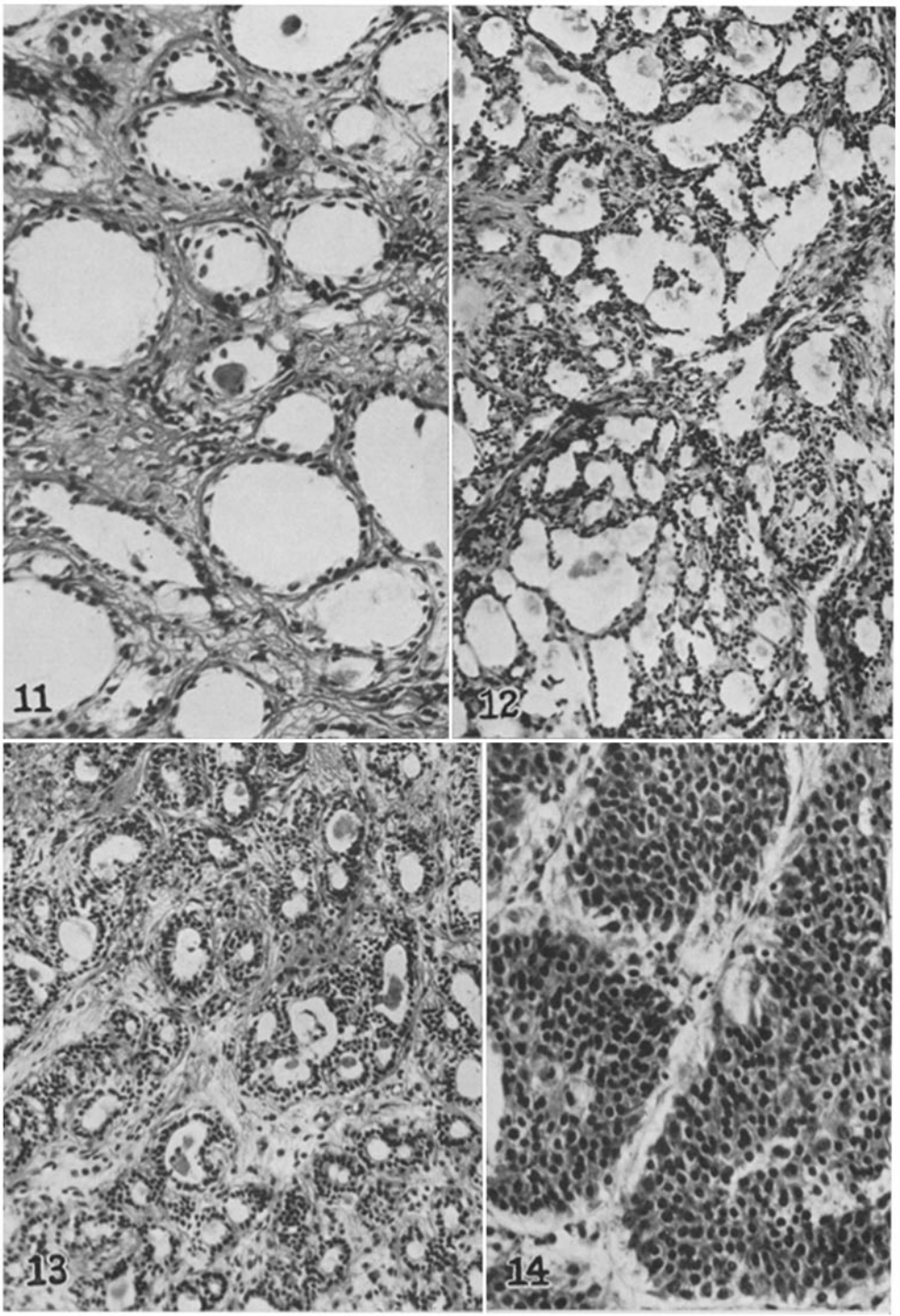
PLATE 8

FIG. 11. Mammary carcinoma which arose in an ovariectomized rat after feeding 3-methylcholanthrene, 5 mg. for 271 days. The acini are lined with flat epithelial cells. × 300.

FIG. 12. Hormone-withdrawal atrophy in a mammary cancer 33 days after removal of the ovaries. The epithelium lining the tumor acini has been reduced to a single layer of small cells. × 300.

FIG. 13. Atrophy in a mammary cancer following the injection of dihydrotestosterone, 1 mg., for 33 days. × 170.

FIG. 14. Histologic appearance of a mammary cancer which progressed despite the injection of dihydrotestosterone, 1 mg. for 33 days. The tumor consists of solid cords of cells. × 200.

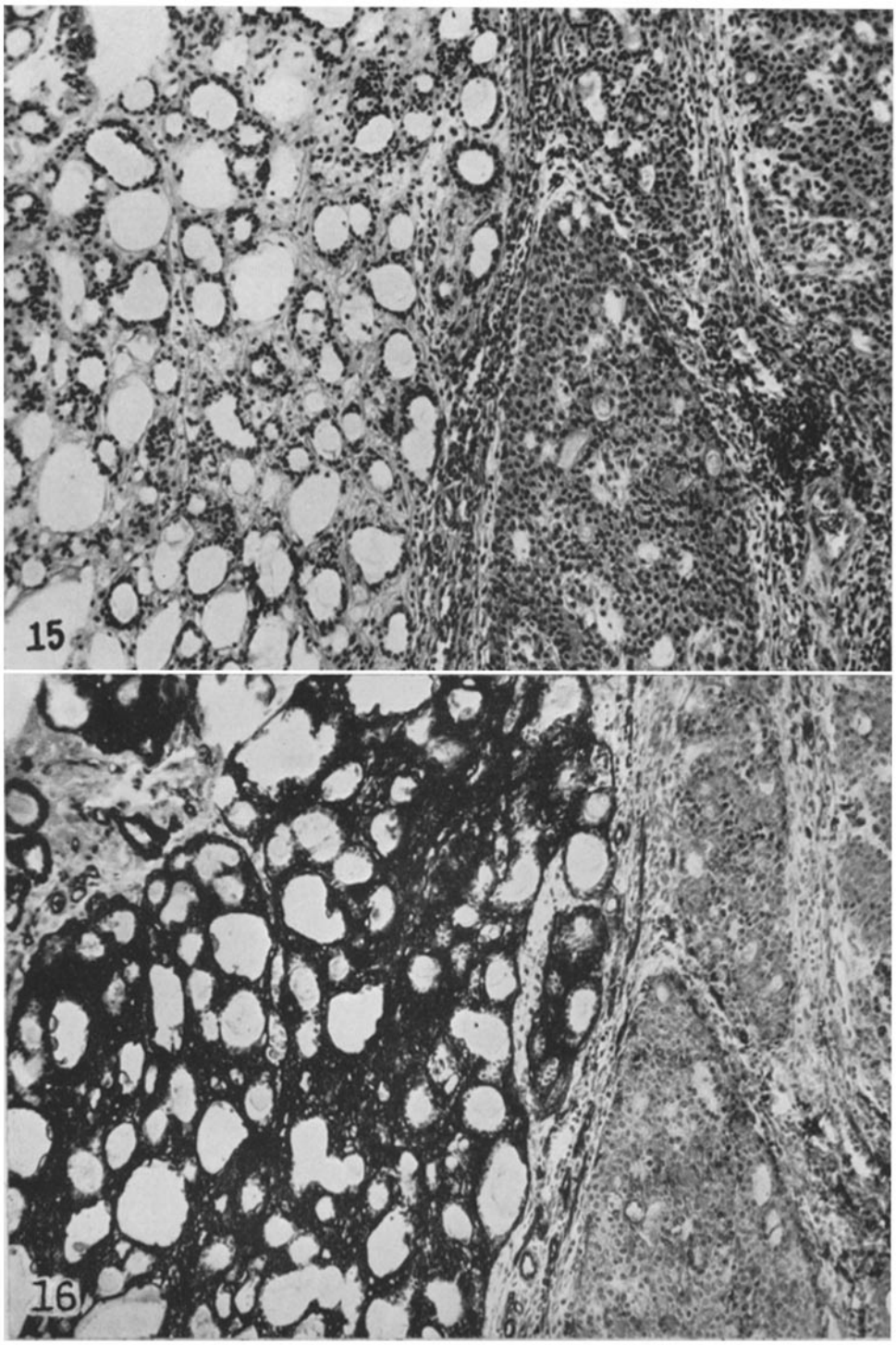


(Huggins *et al.*: Induction of mammary carcinoma)

PLATE 9

FIG. 15. Appearance 33 days after ovariectomy of an induced mammary cancer in the rat showing hormone-withdrawal atrophy (left) adjacent to growth of hormone-independent tumor cells. The atrophic area consists of acini lined with a single layer of epithelial cells similar to those shown in Fig. 11. The hormone-independent cells are similar to those shown in Fig. 14. × 170.

FIG. 16. A section adjacent to that shown in Fig. 15, in which the site of alkaline phosphatase has been demonstrated. An abundance of the enzyme is present in the cells between the atrophic acini (left). × 170.



(Huggins *et al.*: Induction of mammary carcinoma)