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CURRENT PROBLEMS IN RESEARCH

Induction and Extinction of Mammary Cancer

A striking effect of hydrocarbons permits analysis of mechanisms of causes and cure of breast cancer.

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A single feeding (*1, 2*) of any of a number of polycyclic aromatic hydrocarbons (*3*) evokes tumors predominantly of the breast in the albino rat. Under conditions which have been defined (*2*), cancer arises invariably, rapidly, and selectively in the mammary gland. This is a spectacular phenomenon. Now it will be shown that mammary cancers established in this way can be completely extinguished in a considerable proportion, but not in all, of the animals by a modification of their hormonal status. Effects of these sorts brought about by new and facile methods permit analysis of the mechanisms of the causes and extinction of cancer—two of the central problems in cancer research.

Remarkably, a single dose of the polycyclic aromatic hydrocarbons mimics, in its effect, a single exposure to a sublethal dose of gamma radiation (*4*) in the selective induction of mammary tumors in the albino rat.

Induction of Mammary Tumors

Mammary tumors occur spontaneously rather frequently in Sprague-Dawley albino rats (*5*). In our colony, 164 females, virgin and untreated, were observed for 310 days. Eight of these rats developed mammary tumors, cancerous in two animals and benign in the other six. A single dose of an appropriate hydrocarbon accelerates this process and induces tumors of the mammary gland in every animal. A single meal introduces the cancer problem.

In retrospect, it appears that development of the rapid methods under discussion was unduly slow, having required a quarter of a century. Wieland and Dane (*6*) synthesized 3-methylcholanthrene by pyrolysis of deoxycholic acid, with subsequent dehydrogenation of the product. Maisin and Coolen (*7*) repeatedly painted the skin of mice with 3-methylcholanthrene or with benzo(a)pyrene and observed that, in addition to cancer of the skin, mammary cancer arose in 18 percent of the mice after 7 months. Engelbreth-Holm (*8*) painted mice in the same way that the Louvain workers did and found that mammary cancer arose only in females, whereas the males were insusceptible; these experiments demonstrated a hormonal component in the process.

Wilson, DeEds, and Cox were the first to observe that distant tumors arose following the incorporation of aromatic amines in a diet which had been fed for many weeks. In their experiments (*9*) the carcinogen was N-2-fluorenylacetamide, and tumors of liver, bladder, and mammary gland were evoked. Shay (*10*) administered a small dose of 3-methylcholanthrene daily by stomach tube to female rats for many months and observed that many of the rats developed mammary cancer and that it arose after 4 to 12 months; the incidence of mammary cancer was high, but a long time was required for the tumors to become manifest. Next, the time of incidence was accelerated when it was found that mammary cancers were evoked in a few weeks when large but tolerable doses were fed daily to rats (*1*).

But a goal in research on the induction of mammary carcinoma by chemical methods is to induce cancer of the breast rapidly and invariably by a single dose. This goal was attained, and several successful methods were found. In one of these procedures the animals were fed a single large amount of a polynuclear aromatic hydrocarbon. This effect was first demonstrated (*1*) with 3-methylcholanthrene; mammary cancer was evoked in some, but not in all, of the animals. Other carcinogens are far more effective than 3-methylcholanthrene. When a single feeding of 7,12-dimethylbenz(a)anthracene (7,12-DMBA) was given, mammary cancer arose invariably within 21 to 60 days in our laboratory. This is somewhat comparable to the famous experiment of Rous (*11*), who injected a cell-free filtrate of Rous chicken sarcoma I into other fowls and observed the first palpable tumor 10 to 21 days thereafter.

The conditions under which cancer of the breast is evoked by hydrocarbons are highly restricted, but the restrictions are easily bypassed. Six parameters have been defined; these critical factors are the nature and dosage of the hydrocarbon and the species, strain, age, and hormonal status of the recipient. In our experience with more than 700 animals in consecutive investigations, mammary cancer (Fig. 1) developed in all healthy female rats of the Sprague-Dawley strain, age 50 to 65 days, after a single feeding of 7,12-DMBA (20 mg) dis-

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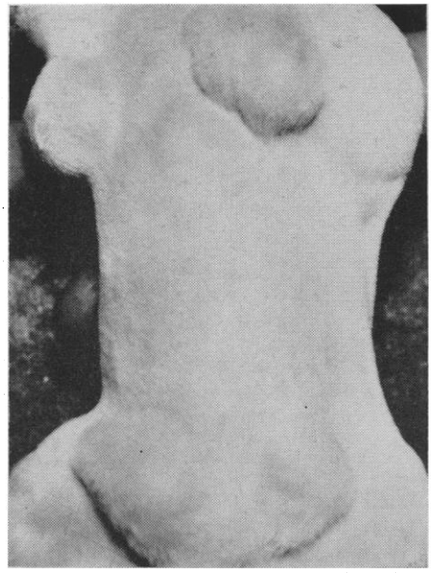


Fig. 1. Multiple mammary cancers in a rat, age 100 days, after a single feeding of 7,12-dimethylbenz(a)anthracene (20 mg) at age 50 days.

solved in sesame oil (1 ml). This method has distinct advantages over multiple feedings in its extreme simplicity, in savings of labor and of rare or costly compounds, in relatively lower exposure of personnel to potentially hazardous substances, and in the speed with which tumors arise. The superficial position of the mammary gland permits ready detection of the cancers by palpation. The end point is sharp in studies of this sort, since the cancers are firm in consistency and discrete. A tumor weighing 8 to 10 milligrams can be detected with ease, but subsequent growth of the tumor and histological examination are required for confirmation. The earliest mammary cancer has been detected by histological search 11 days after the single feeding and by palpation in 20 days, and usually all rats develop mammary cancer within 60 days.

The mammary carcinogens, while differing in molecular constitution, all produce the same types of tumors. One of the most effective carcinogens is 7,12-DMBA, since it is highly active, and a small dose, which is not lethal to the animals, evokes tumors. A single feeding of 7,12-DMBA to 38 rats, observed for 180 days thereafter, evoked tumors as follows: mammary cancer, 100 percent; mammary fibroadenoma, 89 percent; ear-duct tumors, in two rats; leukemia, in one. Tumors of other organs are less commonly observed.

The mammary cancers evoked by aromatic hydrocarbons are all rather similar in cytologic appearance, and all have the cellular pattern of papillary adenocarcinoma. They rarely metastasize but kill the host through attaining great size and through invading adjacent tissues, with consequent hemorrhage and ulceration. In our hands homotransplantation succeeded with approximately 30 percent of the tumors. This low rate of "takes" is in sharp contrast to the rate (100 percent) for transplantation of the benign fibroadenomas. The respiration values (*12*) are similar to those of the normal lactating mammary gland (Q_{O_2}, 12). The high rate of glycolysis ($Q_{Lac}{}^{O_2}$, 12–18), which Warburg found to be distinctive of the metabolism of cancer (*13*), prevailed in the induced carcinomas.

Whereas all the mammary cancers were rather similar in cytologic appearance, there were profound differences in physiologic properties in their growth in response to hormonal status, as discussed later. The hydrocarbons evoke two distinctive types of mammary neoplasms: benign fibroadenoma and cancer. The mammary cancers are at first hormone-responsive. Later, some of the cancers lose hormone responsivity, whereas other cancer cells remain hormone-dependent.

Whereas the administration of 7,12-DMBA by gastric intubation is a highly efficient method of inducing mammary cancer, the compound need not traverse the entire gastrointestinal tract for sufficient absorption of the hydrocarbon to cause cancer of the breast. The injection of 7,12-DMBA (20 mg) into the lumen of the colon was followed by the development of mammary cancer in many rats (*14*). But 7,12-DMBA need not traverse any part of the intestine to evoke cancer. A single intravenous injection of 7,12-DMBA (2.5 mg) prepared as a fine emulsion induced mammary cancer in every rat (*14*).

Moreover, the implantation of a compressed pellet of 3-methylcholanthrene in the spleen was followed by the development of mammary cancer (*2*). The pellets were weighed before implantation and at the end of the experiment; no decrease in the weight of the hydrocarbon occurred in 100 days. An amount of 3-methylcholanthrene too small to be detected on our analytical balance had induced mammary cancer.

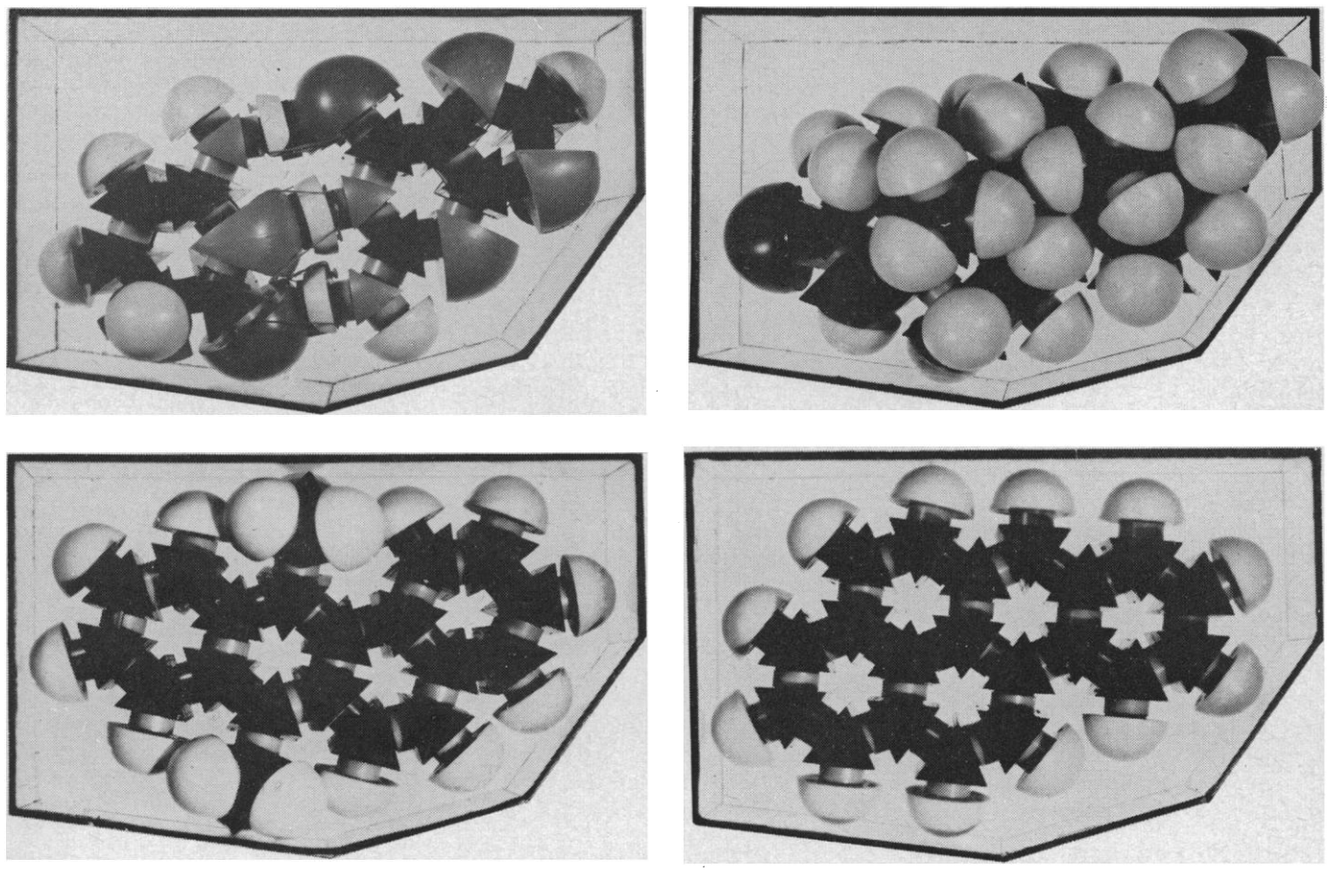
Molecular Characteristics of Mammary Carcinogens

Many substances (Table 1) with divergent molecular structures have in common the property of inducing mammary cancer selectively and rapidly in the rat, and the effect appears to be related to participation in a molecular complex by a polynuclear aromatic hydrocarbon possessing a prerequisite geometric configuration and thickness.

Benz(a)anthracene (I) did not evoke mammary cancer; the addition of one methyl group in either meso- position of this molecule to form 12-methyl-(II) or 7-methylbenz(a)anthracene (III) resulted in a weak carcinogen. The addition of two methyl groups to benz(a)-anthracene to form 7,12-DMBA (IV) resulted in an extremely powerful carcinogen. The presence of methyl groups endowed the molecule, otherwise inactive, with the ability to induce cancer. The addition of a salient alicyclic ring has an effect similar to that of methyl groups—for example the addition of a cyclopenteno ring across the 7 and 8 positions of benz(a)anthracene to give the strongly carcinogenic cholanthrene. Alternatively, the extension of conjugation by the addition of an extra aromatic ring converts benz(a)anthracene to benzo(a)pyrene (V), a highly carcinogenic compound.

Table 1. Induction of mammary cancer by a single feeding of polynuclear hydrocarbons. The compounds, dissolved in sesame oil, were administered by stomach tube to female Sprague-Dawley rats, age 50 days.

No.	Compound	Dose (mg)	Rats (No.)	Mammary cancer (No.)	%
I	Benz(a)anthracene	200	18	0	0
II	12-Methylbenz(a)anthracene	100	12	2	17
III	7-Methylbenz(a)anthracene	100	13	4	31
IV	7,12-Dimethylbenz(a)anthracene	20	700	700	100
V	Benzo(a)pyrene	100	9	8	89
VI	Phenanthrene	200	10	0	0
VII	2-Aminophenanthrene	70	10	10	100
VIII	2,4,7-Trinitro-9-fluorenone	100	20	7	35



Figs. 2–5. Courtauld molecular models in a plastic frame constructed to fit cytosine-guanine. Fig. 2 (top left). Cytosine-guanine. Fig. 3 (top right). Progesterone. Fig. 4 (bottom left). 7,12-Dimethylbenz(a)anthracene. Fig. 5 (bottom right). Benzo(a)pyrene.

Phenanthrene (VI) was not carcinogenic in the experiments reported here, but 2-aminophenanthrene (VII) is a strong carcinogen; the amino group conferred carcinogenicity on the otherwise inactive molecule. The compounds (I-VII) have phenanthrene structure, but an ethylene bridge between aromatic rings is adequate; 4-dimethylaminostilbene synthesized by Haddow (*15*) is a moderately effective mammary carcinogen (*3*), whereas stilbene did not produce cancer.

All of the special carcinogens are flat molecules with conjugated double bond systems and possess substituent groups of a special sort, or (what is equivalent) an additional ring at a salient position in the molecule.

We see that the parent molecular species [benz(a)anthracene; phenanthrene; stilbene; fluorene] are devoid of carcinogenic activity; the potency of aromatic hydrocarbons in inciting cancer depends on the contribution of substituents or an additional ring at a special site. Substituents with effective potency in converting an otherwise inactive molecule to a powerful carcinogen are the methyl and amino groups and their derivatives, dimethylamino and acetylamino. The addition of an extra aromatic ring to benz(a)anthracene to form benzo(a)pyrene creates a compound nearly as effective as 7,-12-DMBA. The common property of $-CH_3$, $-NH_2$, and the salient aromatic rings is their ability to donate electrons to appropriate acceptors as π-donors or N-donors. Szent-Györgyi (*16*) discovered that the carcinogenicity of aromatic hydrocarbons is correlated with their ability to form charge transfer complexes with local acceptors such as I:I. He and his co-workers (*16*) state, "Carcinogenicity of these substances is connected with their ability to form strong charge transfer complexes with local acceptors and give off an electron."

The carcinogenic compounds (II to V, VII) are powerful electron donors which form strong charge complexes with electron acceptors, for example, trinitrobenzene and trinitrofluorenone. It was remarkable to learn that the strong electron acceptor 2,4,7-trinitro-9-fluorenone with melting point of 176-7° (VIII) is also a rather powerful carcinogen, under conditions shown in Table 1. Nitro groups conferred carcinogenicity on the biologically inactive parent compound 9-fluorenone. It is apparent that aromatics that are either *strong electron donors or acceptors can induce cancer.*

But electronic factors per se are not enough to cause cancer. Steric factors are involved. N. C. Yang (*17*) has shown that there is a direct increase in carcinogenicity as aromatic hydrocarbons become sterically similar to steroids.

There is a remarkable similarity in geometric pattern (but not in molecular thickness) between carcinogenic polynuclear aromatic hydrocarbons, growth-promoting steroids, and the base pairs of nucleic acids; this was easily demonstrated. A molecular model was made of guanine-cytosine, and a frame was constructed to surround it (Fig. 2). In this frame similar atomic models of progesterone (Fig. 3), testosterone, and estradiol-17β fit neatly. Similarly, all

mammary carcinogens (*3*) (Table 1) can be inserted within this frame (Figs. 4 and 5). The polynuclear aromatic carcinogens have an identical thickness (3.6A) to the base pairs. The steroids are not carcinogenic vis-à-vis the mammary gland of our colony of Sprague-Dawley rats, and it would appear that they are excluded from mammary carcinogenicity because steroids do not possess planarity. Moreover, the thickness of the steroid molecules (about 5 to 6A) far exceeds that of the base pairs and would preclude intercalation in the double-stranded structure of nucleic acids.

The evidence demonstrates that there are three molecular factors of critical significance determining mammary carcinogenicity in polynuclear aromatic hydrocarbons: (i) the electron transfer factor; (ii) the geometric factor (the configuration must resemble that of steroids and of the purine-pyrimidine pairs of nucleic acid); (iii) molecular thickness (this must not exceed the thickness of the base pairs). In brief, the mammary carcinogens must resemble the base pairs of nucleic acid in geometrical configuration and be able to form molecular complexes.

It is premature to identify the chemical nature of the biological component in the putative charged transfer complex formed in the mammary apparatus by polynuclear aromatic hydrocarbons. Only the essential characteristics of the carcinogens have been demonstrated. These hydrocarbons induce both selective cellular necrosis and selective cancer, but many cells of the body escape these types of injuries; damage, not often followed by cancer, is particularly severe in blood-making organs, whereas pituitary and ovary are undamaged (*2*). A property of 7,12-DMBA which makes it unique among the hydrocarbons which have been investigated is its capacity to induce massive necrosis specifically in two layers of adrenal cortex of adult rats (*18*), with consequent adrenal apoplexy.

The selectivity of damage to the breast with the induction of cancer after remote application of hydrocarbons is remarkable. Not all the many millions of cells of the mammary glands become malignant after an effective dose of the hydrocarbon, and only a small number of tumors arise; after a single large dose of 7,12-DMBA the maximal number of mammary cancers observed in this laboratory is 21 in a single animal. A stoichiometric relation (*2*) exists between dose and number of cancers evoked; the minimum effective amount calls forth only one cancer in each animal. Not all the tumors evoked are cancers—many benign tumors are induced in the mammary gland by hydrocarbons. Clearly, the receptivity of the hydrocarbon by the mammary gland to form cancer is confined to a few cells, and to these only during a restricted physiologic period of life. The hydrocarbons change some of the cells to form cancers, whereas other cells do not go "all the way" to the malignant state, and benign tumors result.

Age is an extraordinary factor in the induction of mammary cancer by polycyclic aromatic hydrocarbons. Whereas cancer was induced invariably when the hydrocarbons were given to rats of age 50 to 65 days under the conditions of the experiment, cancer seldom arose when the compounds were administered to animals over age 100 days (*2*).

Shellabarger (*4*) exposed Sprague-Dawley female rats, age 40 days, to a single sublethal dose (400 r) of whole-body gamma radiation or x-rays and observed that cancer did not arise at

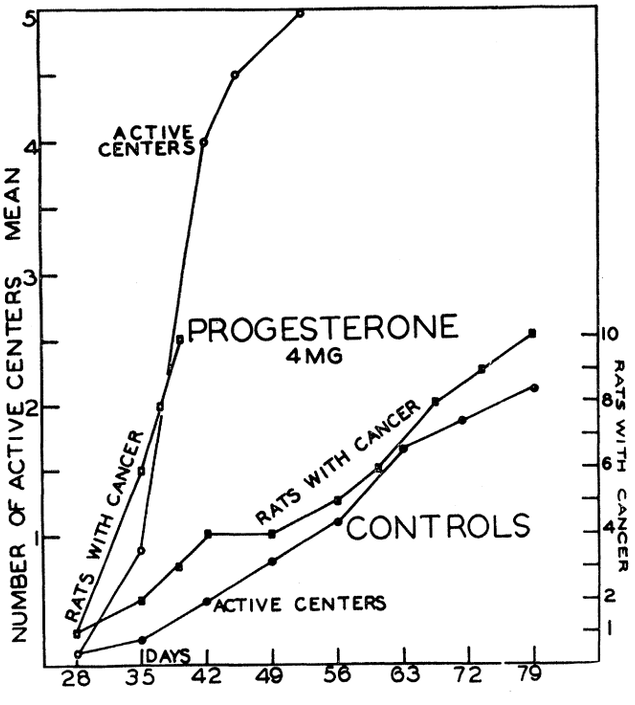


Fig. 6. Development of mammary cancers and increase in their number (active centers) in rats injected with progesterone, as compared with findings for controls not injected with hormones. There were ten animals in each group, and all were fed 7,12-dimethylbenz(a)anthracene (20 mg) on day 0 (age 50 days). One group was injected with progesterone (4 mg) daily from day 15 to day 45.

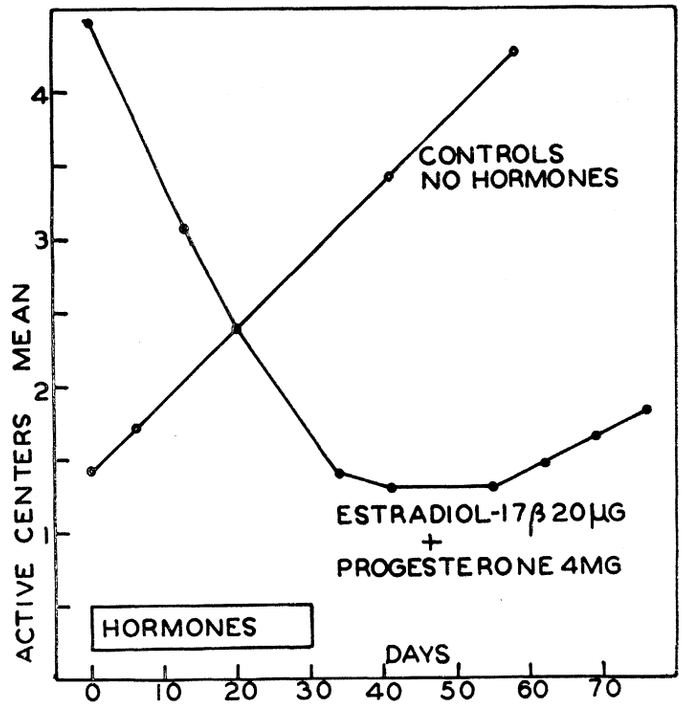


Fig. 7. Decline in the number of cancers (active centers) in rats injected with progesterone plus estradiol-17β for 30 days as compared with a progressive increase in controls that were not injected. All the rats were fed 7,12-dimethylbenz(a)anthracene (20 mg) at age 50 days, and one group was injected with hormones at age 10 days.

random in many tissues but arose preferentially (often exclusively) in the mammary gland. A large number of mammary tumors, both malignant and benign, arose selectively after irradiation. It is highly significant that single shots of radiation and of polynuclear aromatic hydrocarbons evoked the same biologic effect, specifically inducing tumors of the breast in the rat. It is safe to infer that both agents cause selective change in nucleic acids of mammary cells, and that cancer arose from this specific change.

Extinction of Mammary Cancer

The suppression of cancer by chemical means has not been achieved for all neoplasms of all species—far from it—but there have been worth-while results in this area. Progress has been most noteworthy and substantial in the treatment of cancers of endocrine target organs (*19*) and of cancer which produces hormones (*20*).

Regression of mammary cancer, often considerable and long-lasting, has been induced in some hosts, man and animal, by two sorts of alteration of the endocrine status: (i) the withdrawal of hormones essential for the life and growth of the cancers, and (ii) administration of appropriate hormones.

The concept of hormone dependence of cancer rests on the fact that certain cancers derived from endocrine target organs retain growth responsivity in parallel with hormonal status. This principle was found experimentally in a study of the effects of steroids on neoplasms of the canine prostate (*21*) and in a clinical study of prostatic cancer in man (*22*).

In the case of cancer of the breast, discovery of the beneficial effects of steroid withdrawal through oophorectomy (*23*) or adrenalectomy (*24*) emerged directly from bedside observation, since no experimental hormone-dependent cancers in experimental animals were available for study at the time these effects were discovered.

Unlike cancer of the breast of other laboratory animals, one class of mammary cancers of the rat possesses the physiologic characteristic of hormone dependence. These cancers shrink in size after ovariectomy (*1*), but the decrease is temporary and the cancers soon resume their growth. The normal mammary glands of the host undergo atrophy after removal of the ovaries.

Mammary cancers of the rat soon after their induction are highly hormone-responsive. Pregnancy always promotes their growth. Likewise, administration of progesterone to rats possessing ovaries accelerated the appearance of cancers (Fig. 6), increased the number of cancers, and augmented the growth rate of mammary cancer induced by a single feeding of 7,12-DMBA. Moderate or large doses of estradiol-17β (10 to 50 μg daily) delayed the appearance of cancer of the breast, but after the administration of this steroid was discontinued, all of the animals eventually developed mammary cancer.

But the administration of estradiol-17β with progesterone in appropriate doses has a conspicuous effect in that a large number of cancers were destroyed. Estradiol-17β converted, in its biological action, progesterone from an

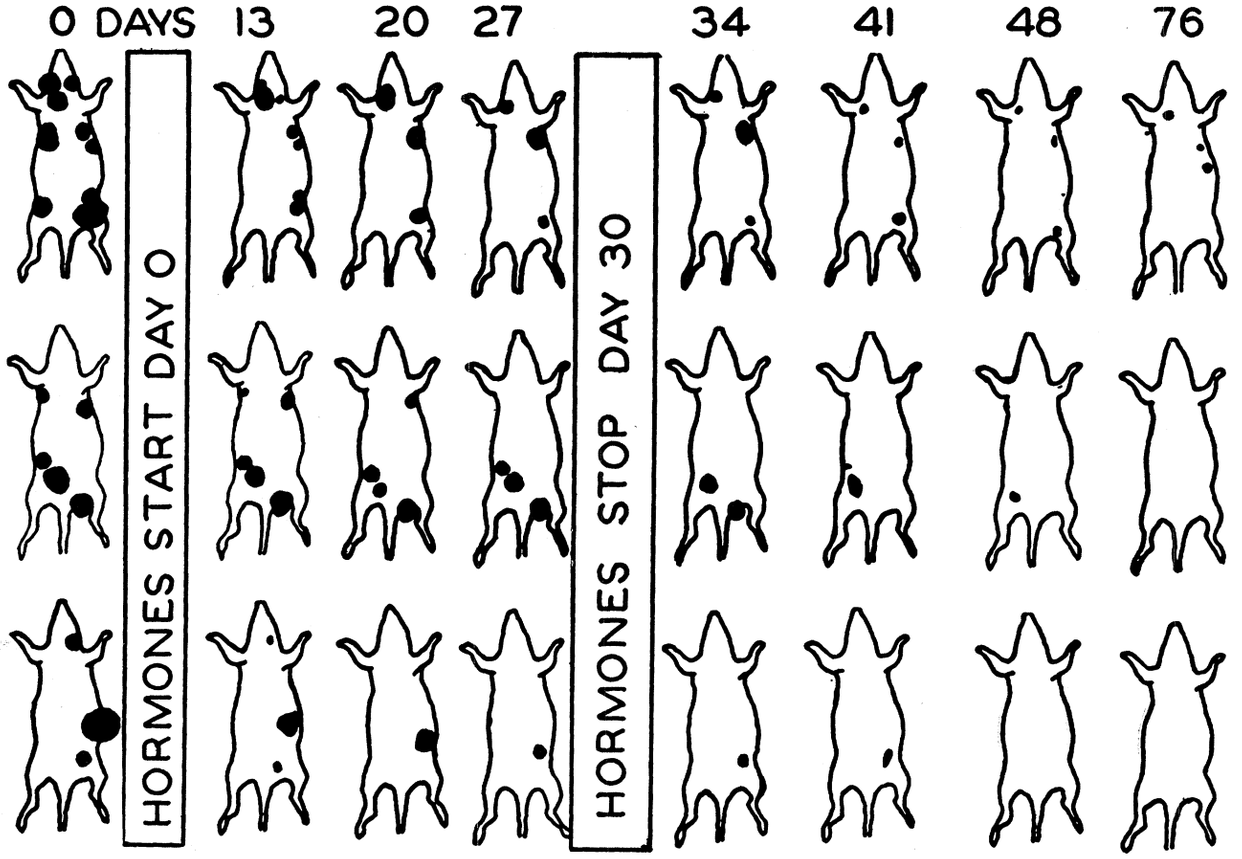


Fig. 8. Decline in numbers and in size of mammary cancers in three rats following injection of hormones [estradiol-17β (20 μg) plus progesterone (4 mg)] for 30 days, starting 50 days (day 0) after a single feeding of 7,12-dimethylbenz(a)anthracene (20 mg), which had been given at age 50 days.

enhancer of breast cancer to a suppressor. In an experiment (*25*), susceptible female rats were fed a single dose of 7,12-DMBA (20 mg) at age 50 days; at age 65 days, estradiol-17β (20 μg) and progesterone (4 mg) were administered concurrently, and they were administered each day thereafter for a period of 30 days; the rats were not treated for the remaining 180 days to necropsy. In a group of 100 rats treated in this way, 52 animals were free from cancer of the breast at necropsy at the end of the 6-month period of observation, whereas all of their control companions (fed 7,12-DMBA but not treated with hormones) had succumbed to cancer. Of the 52 rats free from mammary cancer, benign tumors of the breast were present in 16 and ear-duct cancer in two. Mammary cancers were the only tumors which had been destroyed by the concurrent administration of estradiol-17β and progesterone.

The experiment was repeated under more rigorous conditions, with rats with large mammary cancers. At age 50 days, 7,12-DMBA was fed to 35 rats, and at age 100 days all of the recipients had multiple mammary cancers. As in the previous experiment, estradiol-17β and progesterone were administered for the limited period of 30 days, and the animals thereafter were observed without further treatment for 6 months. In the rats injected with progesterone plus estradiol-17β, there was a decrease in the number of cancers observed (Fig. 7) and tumors were completely extinguished in ten rats (29 percent) (Fig. 8). This is the only method known at the present time by which experimental mammary cancer has been completely extinguished.

The concurrent administration of estradiol-17β and progesterone in the stated amounts results in extremely hyperplastic mammary glands and a depression in ovarian weight. But the function of the ovaries recovered rapidly after termination of the hormone injections; the first estrus occurred 6 to 9 days later, and rats in one group were mated within 2 weeks after the last injection of hormones and underwent normal pregnancy followed by normal delivery. Thus, soon after the termination of the hormone treatment, the animals attained an endocrine status highly conducive to the growth of mammary cancer, but no cancers ensued during the subsequent 6 months because of their extinction by the earlier administration of the hormones.

The response of hormone-dependent cancers to the removal of supporting hormones differs in an essential manner from that of the normal cells from which they originated. Normal hormonal target cells survive and merely decrease in size and metabolic activity after hormones have been withdrawn, but the highly hormone-dependent cancer cells in the host die as a result of hormone deprival. Therefore, in cancers of this dependent class the hormones are of critical importance for the life of the cell. In the experiments under discussion a significant proportion of mammary cancers was destroyed, not by hormone withdrawal, but by the administration of two hormones normally produced in the ovary and adrenal; one of these (progesterone) given singly accelerated vastly the cancerous process in intact rats, whereas the other (estradiol-17β) merely depressed the growth rate of mammary cancer. Together these hormones produced very vigorous growth of normal mammary glands, while completely destroying many of the cancers of the breast. It would appear that the hormones in combination had interfered with that steroid hormone mechanism which is of paramount importance for the life of certain mammary cancers. Hormone interference is a novel principle in the extinction of cancer (*26*).

Note added in proof: Haddow [*Canadian Cancer Research Conference*, R. W. Begg, Ed. (Academic Press, New York, 1957), p. 361] raised the question "whether there is any significant association between the molecular planarity which is a feature of the carcinogenic hydrocarbons (and the absence of which apparently entails biological inactivity) and the flatness of the nucleotide plates. The purine : pyrimidine bonded pairs of the Crick-Watson model present planar structures of the same general order of size as the hydrocarbons and it is a question whether these thoughts should not be further pursued."

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