The earliest indication that regression of advanced cancer in living creatures can be effected was derived from removal of normal organs, namely, the ovaries of women (2). This empiric therapeutic procedure was remarkable insofar as it was made before there was any concept of endocrine action. Following this discovery, Lathrop and Loeb (32) found that removal of ovaries prevented onset of mammary cancer in a considerable number of mice. Lacassagne (30) was the first to show that a correlation exists between development of cancer and hormones, since a steroid with estrogenic activity incited formation of mammary cancer; in a famous experiment weekly injections of 30 μg of estrone benzoate evoked cancer of the breast in each of 3 male mice in 5–6 months.

The proof that modification of hormonal status can cause regression of cancer came from a study of tumors of canine prostate (15): injection of stilbestrol resulted in a profound decrease in size of benign and malignant prostatic tumors, even those of huge size, in the dog. The hormonal control of cancer applied to far advanced carcinoma of the prostate in man (21). It was found that testosterone accelerated the growth of human prostatic cancer, whereas, in contrast, orchiectomy or the injection of estradiol benzoate or of stilbestrol caused in most patients a dramatic and long-lasting regression of their cancers. Stilbestrol was the first synthetic substance found to be an anti-cancer drug.

There are 2 principles in the destruction of cancer cells in living creatures by modifications of endocrine status: (a), hormone deprival; (b) hormone interference with large amounts of critical compounds.

The experience with prostatic tumors led to the concept of hormone dependence and independence. This terminology (24) was first used in 1945. There is a fundamental difference between a normal hormonal target cell and its hormone-responsive malignant derivative. In a normal cell of origin supporting hormones act as catalysts of growth and metabolism, but these compounds are not essential for life of the cell. In contrast, a hormone-dependent cancer cell dies when supporting hormones are withheld or their source is removed. In consequence, a cancer cell cannot participate in growth cycles (13) characteristic of the normal cell of origin created by alternately administering and withholding supporting hormone. In hormone-dependent cancers of all sorts, prostatic and others, the supporting hormones are of cardinal importance in maintaining the life of the malignant cell. This is the principle of cancer control by hormone deprivation.

Cancers can also be controlled by supplying large amounts of hormones; this is the novel principle (22) of hormone interference, a pharmacologic concept. Two cases where hormone interference kills cancer cells will be cited.

1. Heilman and Kendall (10) administered large amounts of cortisone to mice bearing a transplanted lymphosarcoma: “Although dramatic and apparently complete cures are produced, they are only temporary in a majority of the animals.” Only lymphomas of adult male mice failed to be resorbed when cortisone was given, and the combined administration of cortisone plus estradiol-17β caused rapid regression of the tumors in these males. Whereas corticosteroids exerted lethal effects, adrenalectomy does not cause regression of lymphomas in mice.

Pearson et al. (38, 39) observed that ACTH or cortisone

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resulted in temporary regression in human lymphatic leukemia and Hodgkin's disease.

2. Huggins et al. (22, 25) induced mammary carcinoma in rats, which were then treated for a limited time with large amounts of estradiol-17β plus progesterone. This combination of hormones excited such exuberant growth of normal mammary cells that the breast resembled that of rats late in pregnancy. Nevertheless, many of the mammary cancers were completely extinguished (25), and 52% of the rats were free from cancer 6 months after steroids were discontinued. Landau et al. (31) found that "a combination of 50 mg. of progesterone and 5 mg. of estradiol benzoate injected intramuscularly and daily, induced measurable and clinically worth-while improvement in 9 of 15 patients, including 1 man, with disseminated mammary cancer. Benefit was usually obtained in patients in whom other forms of endocrine therapy such as adrenalectomy and oophorectomy had previously promoted tumor regression." Mammary cancer can regress from either hormone deprival or hormone interference from excess of hormones.

At the present time 7 sorts of cancer are known to be responsive to hormonal modifications of the milieu intérieur since shrinkage of cancer and betterment of its host follow changes in his hormonal status. Cancers of this sort are of the breast, prostate, thyroid (1), endometrium (27), kidney (4, 29), and seminal vesicle (26), and also include lymphoma and leukemia.

In a great many clinical patients, men and women, mammary cancer regresses after any of a considerable number of hormonal modifications. The regression so induced can be profound and long lasting (6, 11), and, accordingly, the treatment is of value to the patient. With a single exception (22), all of the procedures found to be beneficial for human cancer of the breast were found directly at the bedside through clinical investigation. The vast amount of work in the laboratory on mammary cancer has yielded little that has been applied as therapy for women with cancer of the breast.

Hormone-dependent experimental mammary cancer.— Spontaneous mammary cancer is common in the dog and mouse as well as in man. In the dog mammary cancers did not shrink after ovariectomy or adrenalectomy (23). In various strains of mice possessing the milk agent, spontaneous mammary cancers of palpable size did not regress following ovariectomy, hypophysectomy, or administration of testosterone (35). In this regard the tumors of the dog and mouse differ from many human mammary cancers. It was necessary to find a hormone-dependent experimental mammary cancer.

The key to the solution of this problem was recognition of a species with hormone-responsive cancers (14). So far as is known, the rat is unique among the species available for research on breast cancer, since only in this species are mammary cancers hormone dependent. Hormone-dependent mammary cancer now can be produced invariably and in potentially unlimited supply with methods of extreme simplicity.

There are 3 methods for induction of mammary cancer in rats: exposure to (a) estrogens, (b) ionizing radiation, and (c) aromatics. Aromatics are the most efficient, since they give the highest yield of mammary carcinomas in the largest percentage of rats.

Mammary carcinoma induced by estrogens.—The continued exposure to critical doses of estrogens leads to mammary cancer. The method is slow and inefficient, since many months elapse before the tumors appear and many of the animals never develop breast cancer (37). Noble and Collip (36) found that the implantation of estrone pellets into random-bred hooded rats was followed by the development of adenocarcinoma in 28 of 49 rats, the first tumor appearing after 226 days. Maisin et al. (34) implanted pellets of stilbestrol in rats that were observed for more than 300 days; no mammary cancers arose. We injected 50 μg of estradiol-17β daily for 400 days in 15 female Sprague-Dawley rats beginning at age 66 days, the rats earlier having been subjected to ovariec-tomy-hysterectomy. One rat developed mammary carcinoma, which was detected after 358 days; 14 rats remained free from breast cancer.

Mammary carcinoma induced by ionizing radiation.— The selective induction of mammary cancer in the rat by irradiation was discovered by Hamilton (9). A single dose of radioac-tive isotopes, X-rays, or γ-irradiation elicits mammary tumors, which arise rather quickly, but in no series have all irradiated rats developed mammary cancer.

Hamilton et al. (9) observed a 40% incidence of mammary cancer in female S-D rats, aged 55 days, after a single injection of 32P. Maisin et al. (34) found that some young female rats (strain unspecified) developed mammary cancer after total-body irradiation with X-rays. In S-D female rats, aged 52 days, given single total-body X-irradiation (400 r) and observed for 6 months, mammary carcinomas had the following characteristics (17): incidence in 29.7% of the rats; detection of 92% of the cancers within 70 days; multiple carcinomas in 18% of the rats. In experiments of Cronkite et al. (5) 79% of S-D female rats developed mammary tumors, benign and malignant, within 10 months after exposure to a single dose of total-body X-irradiation (400 r) and the 1st tumor was detected 41 days after the exposure. Shellabarger et al. (43) found that 56% of female S-D rats, aged 40 days, developed mammary tumors after a single total-body γ-irradiation with a 60Co irradiator at age 40 days.

Mammary carcinoma induced by aromatics.—Maisin and Coolen (33) painted the skin of mice repeatedly with a solution of 3-MC and found, in addition to skin cancer, that carcinoma of the mammary gland arose in 18% of the mice after 7 months. Wilson et al. (47) discovered that incorporation of an aromatic (2-AAF) in the diet of rats induced cancer in various tissues; mammary cancer arose in a small number of the animals. Shay et al. (42) observed that the daily intragastric instillation of 3-MC, 2 mg, for many months induced mammary cancer in a large percentage of Wistar rats; the tumors were detected after 129-383 days.

We found out (14, 18) that a single large but tolerable dose of any of a considerable number of aromatics in the rat consistently induces mammary cancer selectively and rapidly. Although the conditions for induction are highly
restricted, they are easily satisfied. Eight parameters have been identified in induction of mammary cancer. These are (a) the nature and (b) dose of the aromatic; (c) species; (d) strain (44); (e) age (16); and (f) the hormonal status of the rat; additionally, (g) the animals must remain free from infectious disease and (h) have no contact with cancer-protective substances (20).

In capsule, mammary cancer has always developed in our laboratory in 1500 consecutive female rats of S-D strain, aged 50 days, given a single i.v. injection of a lipid emulsion, 1 ml, containing 7,12-dimethylbenz(a)anthracene, 5 mg; the animals were kept in metal cages in an air-conditioned room at 25° ± 1°C. A single feeding by gastric instillation of a solution of vegetable oil, 1 ml, containing 7,12-DMBA, 20 mg, can substitute for the lipid emulsion. The earliest mammary cancer induced by this technic was detected by palpation 20 days after 7,12-DMBA was given. In 90 consecutive rats (12) mammary cancer was detected in 28–92 days, mean 42.8 ± 11 days.

The mammary gland of the young adult S-D female rat stands in the forefront of cells of living creatures in its susceptibility to induction of cancer. It is equaled only by cells of certain chickens when they are inoculated with a single agent, Rous sarcoma virus I. In a famous experiment of Rous (41), cell-free filtrate of a sarcoma injected into other fowls evoked tumors of palpable size after 10–21 days.

Dao et al. (8) fed female rats a single dose of 7,12-DMBA and after 4 hr transplanted their mammary glands to other rats; mammary cancer arose in the homologous recipients of the grafts.

Many aromatics share in common the ability to induce mammary cancer in the rat after a single dose. In this regard, from a molecular standpoint, 4-aminodiphenyl, consisting of 2 rings, has the simplest structure of the aromatic amines. The least complex of polynuclear aromatic hydrocarbons, a monomethylbenz(a)anthracene (7-methyl- or 12-methyl-), consists of 4 rings. But in efficiency of dosage, 7,12-DMBA exceeds all other aromatics by an order of magnitude.

Nature of aromatic-induced tumors in the rat.—A single feeding of 7,12-DMBA, 20 mg, to 38 female S-D rats, aged 50 days, and observed for 180 days thereafter evoked tumors (25) as follows: mammary cancer, 38 rats; mammary fibroadenoma, 34 rats; ear duct carcinoma, 2 rats; leukemia, 1 rat. Tumors of other structures are less common. The tumors occur in 2 discontinuous series: mammary carcinoma manifests itself early, and other tumors, after 3 months.

All of the mammary cancers evoked by aromatic hydrocarbons are rather similar in cytologic appearance, and all possess the cellular pattern of papillary adenocarcinoma; mitoses are abundant. Often in some of the tumors islands of squamous carcinoma are present (50). The mammary cancers rarely metastasize but kill the host by invading muscle and skin, with consequent hemorrhage and ulceration. The respiration values (40) are similar to those of the normal lactating mammary gland; the high rate of glycolysis, which Warburg (46) found to be distinctive of the metabolism of cancer, prevailed in the induced carcinomas.

Young and Cowan (49) found that a number of the induced mammary carcinomas of the rat undergo spontaneous regression. Such tumors have these characteristics: mammary epithelium remains cubical or columnar; many mitotic figures are present; stroma of the tumor becomes heavily infiltrated with lymphocytes and
other mononucleated cells. In our laboratory, we have not observed spontaneous regression of all of the cancers in any rat bearing multiple mammary cancers. It would appear that spontaneous regression is dependent on the quality of the individual tumor and is not a function of the host's immunologic status.

**Hormonal prerequisites for induction of mammary cancer in the rat.**—The physiologic state of the mammary tree, determined by endocrine effects, is of critical significance in induction of mammary cancer. Repeated feeding of 3-MC to groups of rats evoked mammary cancer in all intact females and in no hypophysectomized mate (19). Young (48) fed 3-MC to hypophysectomized rats treated with estradiol-17β, progesterone, and bovine growth hormone; mammary carcinoma arose in 5 rats in a group of 9.

**Hormonal influence on mammary cancer of the rat.**—Changes in hormonal status have little or no influence on hormone-independent mammary cancers. But the majority of mammary carcinomas in the rat are profoundly influenced by endocrine factors, and after appropriate changes in hormone status their growth can be accelerated or retarded, and indeed many of the growths can be extinguished.

Acceleration of growth of the mammary cancers occurs in pregnancy (7, 22); in pseudopregnancy (7); and following administration of progesterone or 9α-bromo-11-ketoprogesterone (19).

Retardation of growth of mammary cancers and, at times, tumor extinction occur after ovariec-tomy (14) or hypophysectomy (19, 28); the relative effectiveness of these 2 ablative procedures is shown in Chart 1. Moreover, a severely deleterious effect on mammary cancer follows administration of testoste-rone (14), estradiol-17β with progesterone (25), or equine gonadotropin (19).

Bielschowsky (3) found that mammary cancer of the rat frequently regressed during lactation, whereas ovariec-tomy had no effect on growth of the tumors.

The mammary cancers that diminish in size after removal of the pituitary or ovaries have a distinctive cytologic appearance. Many of the carcinoma cells are destroyed. In the remaining tumor acini the many layers of plump epithelial cells are replaced by a single layer of flat cells (14); the most obvious and consistent changes are flattening of the epithelium and an increase in size of the acinar lumina (50). Tumors unaffected by hormonal modification retain many layers of epithelial cells.

The inhibitory effect of ovariec-tomy on the development of mammary cancer in the rat was overcome by a daily injection of small amounts of estradiol-17β (14). It has been reported (45) that injection of growth hormone with prolactin elicited growth of tumors in rats with mammary tumors treated earlier with ovariec-tomy.

In the experiments of Kim and Furth (28) the growth of aromatous induced mammary carcinoma was inhibited by ovariec-tomy (72%) and by hypophysectomy (87%). In their hypophysectomy series, tumors could not be identified at autopsy in 37% of the animals. Grafts of functioning pituitary tumors into hypophysectomized rats whose tumors had undergone considerable regression caused the resumption of progressive tumor growth with the reappearance of many new mammary tumors.

From the evidence that has been presented it would appear that both steroids and protein hormones are of significance in the maintenance of mammary carcinoma.

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Two Principles in Endocrine Therapy of Cancers: Hormone Deprival and Hormone Interference

Charles Huggins


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