days, the culture was green and thriving. This strain has been isolated and designated *Chlorella pyrenoidosa* C-37-2-G4. The modified strain grows well in the presence of progesterone. Cultures of the modified strain to which progesterone has been added deteriorate at a much slower rate than those cultures of the original strain to which androstenedione or dehydroisoandrosterone had been added. Furthermore, thin-layer silica-gel chromatography indicates that metabolic products from progesterone are formed by this modified strain.

The incubation of dehydroisoandrosterone for 432 hr with *Chlorella pyrenoidosa* C-37-2 produced at least four different compounds. These were detected by means of thin-layer silica gel chromatography. From the position of the products on the chromatograms, it is probable that one of these was 5-androstene-3β,17β-diol while another was an hydroxylated 5-androstene-3β,17β-diol. The structures of all the indicated conversion products and the possible transformation of steroids by other species and strains of algae are being investigated currently.

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*EXTINCTION OF EXPERIMENTAL MAMMARY CANCER, I. ESTRADIOL-17β AND PROGESTERONE**

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Communicated January 12, 1962

This paper is concerned with extinction of cancer of the breast of rats by hormones which simultaneously induced exuberant growth of the normal mammary glands. Two methods were found to be highly effective in this regard: (1) administration of estradiol-17β together with progesterone and (2) strong stimulation of the ovary with gonadotrophin.

The experiments were made possible by a chemical method[1] of induction of mammary cancer which evoked this tumor invariably in albino rats. The technique is of extreme simplicity. In brief, the rats received a single feeding of 7, 12-dimethylbenz(a)anthracene.[2] Mammary cancer always arises within a few weeks after this

solitary feeding. The conditions under which cancer of the breast is evoked by polynuclear aromatic hydrocarbons are highly restricted but the restrictions are easily obviated; mammary cancer develops in all normal female rats of the Sprague-Dawley strain, age 50–65 days, after a single feeding of 7,12-DMBA, 20 mg, dissolved in sesame oil[1, 3] and otherwise untreated.

The effective suppressive hormonal methods decreased the incidence of mammary carcinoma in all of the animals and destroyed the cancers in about one half of the rats. Only mammary cancers were eliminated. Benign tumors of the breast and ear duct cancer induced by the hydrocarbon were not extinguished.

Experimental.—The experimental animals were normal virgin female Sprague-Dawley rats, kept in air-conditioned rooms and fed a commerical ration. The animals remained free from respiratory disease.

Steroids[4] were dissolved in alcohol and diluted with sesame oil to make the final alcoholic concentration 10 per cent. The solution (0.2 ml) was injected intramuscularly. Equine gonadotrophin was dissolved in 0.15 *M* sodium chloride and the solution (0.5 ml) was injected subcutaneously; 1 unit as defined by Cartland and Nelson[5] was given. Hormones were injected 6 days each week. Throughout this paper, dosage relates to the amount given each day.

A refined sample of 7,12-DMBA, mp 122–3°, was dissolved in sesame oil with the aid of gentle heating, and the container was protected from light thereafter. At age 50 days, 7,12-DMBA, 20 mg, dissolved in sesame oil, 1 ml, was fed to the rats by stomach tube as a solitary dose of this compound. Always a group of these rats was kept as controls. Other groups received injections of hormones for a limited period from age 65–95 days. Estradiol-17β and progesterone were injected together in a single solution. The rats were untreated following the cessation of the hormone injections. The animals were examined for tumors by palpation daily. Mammary cancers visible in the gross at necropsy are designated active centers. The mean number of active centers is the total number of mammary cancers divided by the number of rats in that group. The time of appearance of tumors is dated from the feeding of 7,12-DMBA. All tumors were verified by histological examination. Vaginal smears were obtained by means of a pipette and saline.

Results.—Sprague-Dawley rats are subject to a high incidence of spontaneous mammary tumors.[6] In our colony of control untreated virgin females of this strain receiving no polynuclear aromatic hydrocarbons and observed for 10 months (Table 1), the tumor incidence was: mammary cancer 1.2%; mammary fibro-

TABLE 1

INCIDENCE OF NEOPLASMS IN FEMALE RATS FED 7, 12-DMBA AND THEIR CONTROLS

Tumors	Number	Per cent	Time of detection (days) Range	Median	Mean
a. 164 Control rats received no 7, 12-DMBA					
Mammary cancer	2	1.2	53 & 101	—	—
Mammary fibroadenoma	10	6.1	137–308	278	267 ± 53
Lymphosarcoma	1	0.6	120	—	—
Ear duct cancer	0	—	—	—	—
b. 38 Rats were fed 7, 12-DMBA, 20 mg					
Mammary cancer	38	100	24–61	37	39 ± 6
Mammary fibroadenoma	34	89	147–209	185	194 ± 38
Leukemia	1	2.5	147	—	—
Ear duct cancer	2	5	146 & 246	—	—

A single feeding of 7, 12-DMBA was given to one group at age 50 days. The animals were otherwise untreated. They were observed until age 310 days.

adenoma 6.1%; lymphatic tumor 0.6%. In sisters fed 7,12-DMBA, 20 mg, and observed for 310 days, the tumor incidence was: mammary cancer 100%; mammary fibroadenoma 89%; ear duct cancer 5%; leukemia 2.5%. No other neoplasms were found.

Now let us consider the effects of hormones administered beginning 15 days after a single feeding of 7,12-DMBA.

Effect of pregnancy and progesterone: Both pregnancy and the injection of progesterone alone vastly accelerated the growth of mammary cancer; the incidence of cancer of the breast in each hormonal state was 100 per cent. When pregnancy was induced at age 65 days, the time of appearance of cancer of the breast was shortened and the number of active centers increased in comparison to mammary cancer in virgin females (Table 2). In earlier work, Dao and Sunderland[7] observed that

TABLE 2

INFLUENCE OF PREGNANCY, PROGESTERONE, ESTRADIOL-17β AND GONADOTROPHIN ON INCIDENCE OF MAMMARY CANCER

Endocrine Status	Number of rats	Rats without Cancer		Time of Appearance of Mammary Cancer (days)			Active Centers (mean)
		Number	Per cent	Range	Median	Mean	
Intact controls	48	0	0	27–72	39	41 ± 8	2.7
Pregnancy	34	0	0	23–36	30	30 ± 3	3.8
Progesterone, 4 mg	48	0	0	24–49	32	33 ± 5	3.6
Estradiol-17β, 20 μg	31	0	0	60–111	78	81 ± 14	1.8
Estradiol-17β, 20 μg plus Progesterone, 4 mg	100	52	52	62–182	129	124 ± 31	0.8
Gonadotrophin, 1 U	20	6	30	32–175	88	90 ± 44	1.0

±, Standard deviation.
All rats received a single feeding of 7, 12-DMBA at age 50 days. One group was bred at 65 days. Groups receiving hormones were injected daily between age 65–95 days. The animals were observed for 180 days.

pregnancy and pseudopregnancy decreased the latent period of appearance of mammary cancer in rats repeatedly fed 3-methylcholanthrene.

Progesterone, 4 mg, was a profound accelerator of growth of the induced mammary cancer. The incidence of cancer of the breast was 100 per cent; the time of appearance of the tumors was short; the number of active centers was large (Table 2). In these effects, progesterone, 4 mg, mimicked the tumor-stimulating action of pregnancy.

Effect of estradiol-17β: The appearance of mammary cancer was delayed in all rats injected between age 65–95 days with estradiol-17β, 20μg (Table 2). Fewer active centers were present than in companions not treated with hormones. The time of onset of mammary cancer was prolonged, and its growth was depressed in comparison to females not injected with hormones but the tumors were not extinguished. The incidence of mammary cancer was 100 per cent.

Effect of estradiol-17β with progesterone: The concurrent injection for 30 days of estradiol-17β, 20μg, and progesterone, 4 mg, resulted in a decreased incidence of mammary cancer; 52 per cent of the rats (Table 2) treated in this way were apparently free from cancer of the breast six months after feeding 7,12-DMBA, whereas all of their companions uninjected with the hormones had succumbed with mammary cancer. In these 52 rats, free from mammary cancer, mammary fibroadenomas were present in 16 (31%) and ear duct cancer in 2 (4%). In the 48 rats which had developed mammary cancer, there was a small number of active centers (Table 2).

Since progesterone alone accelerated the growth rate of mammary cancer but

when injected with estradiol-17β was effective in extinguishing cancer of the breast, it was necessary to learn the effective dose of estradiol-17β which converted progesterone from an accelerator to a suppressor of mammary cancer; *that effective dose is estradiol-17β, 20 μg.* In this experiment, progesterone, 4 mg, was injected in all rats with various groups receiving supplementary estradiol-17β in graduated amounts. Supplemental estradiol-17β, 0.1–1 μg, did not abolish the cancer-stimulating effect of progesterone (Table 3), while a dose of 10 μg delayed some-

TABLE 3

INFLUENCE OF INCREASING AMOUNTS OF ESTRADIOL-17β ADMINISTERED WITH PROGESTERONE ON MAMMARY CANCER

Estradiol-17β Dosage (μg)	Number of rats	Rats without cancer	Time of Appearance of Mammary Cancer (days) Range	Median	Mean	Active Centers (mean)
0.1	10	0	25–43	31	32 ± 5	3.7
1	10	0	29–93	35	51 ± 8	1.5
10	10	1	92–210	141	146 ± 43	1.4
20	10	6	102–129	116	118 ± 7	0.6
50	10	6	130–162	133	141 ± 9	0.6

±, Standard deviation.
All rats received a single feeding of 7, 12-DMBA at age 50 days. Hormones were injected daily between age 65–95 days. *All rats received progesterone, 4 mg, in addition to estradiol-17β.*

what the onset of tumor. Estradiol-17β, 20–50 μg (these amounts were equivalent in their action), abolished mammary cancer in many rats and postponed the development of cancer in the remainder, the number of active centers being greatly reduced in the rats with cancer.

How long must estradiol-17β, 20 μg, with progesterone, 4 mg, be given to extinguish mammary cancer? Administration of the hormones for 10 or 20 days decreased the incidence of cancer of the breast but less effectively than treatment for 30 days (Table 4).

TABLE 4

INFLUENCE OF TIME OF ADMINISTRATION OF ESTRADIOL-17β, 20 μG, AND PROGESTERONE, 4 MG, ON MAMMARY CANCER

Time of administration	Number of rats	Rats without cancer	Time of Appearance of Mammary Cancer (days) Range	Median	Mean	Active Centers (mean)
10 days	10	3	69–143	81	81 ± 36	1.3
20 days	10	3	51–168	85	96 ± 45	0.8
30 days	10	5	108–182	141	143 ± 38	0.6

±, Standard deviation.
All rats received a single feeding of 7, 12-DMBA at age 50 days. Hormones were injected beginning at age 65 days.

Effect of gonadotrophin: Equine gonadotrophin, 1 unit, was injected for 30 days in 20 rats. Six (30%) of these rats were free from mammary cancer six months later (Table 2). Large doses of equine gonadotrophin administered for 30 days resulted in great and cystic enlargement of the ovaries;[1] the mammary glands were hyperplastic, orange in color with marked epithelial proliferation consisting of solid and luminated tubules, some of which contained milk.

Effects of estradiol-17β and progesterone on the mammary glands: The growth status of mammary glands was determined quantitatively by measuring their content of alkaline phosphatase and by histologic sections.

The virgin mammary glands of rats age 50–100 days consist of branching systems

of small luminated ducts which terminate in solid nests of end-bud cells. A single feeding of 7, 12-DMBA did not alter this basic pattern except where tumors were induced; the alkaline phosphatase content of the mammary glands of rats which had received the hydrocarbon did not differ from that of untreated mates (Fig. 1).

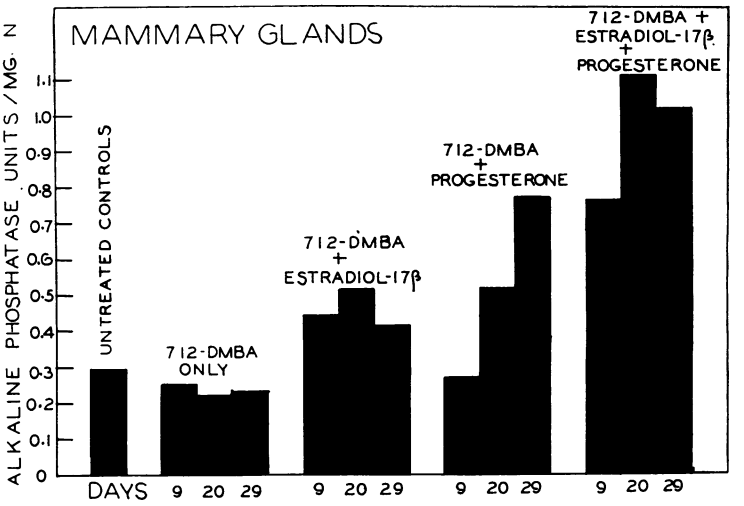
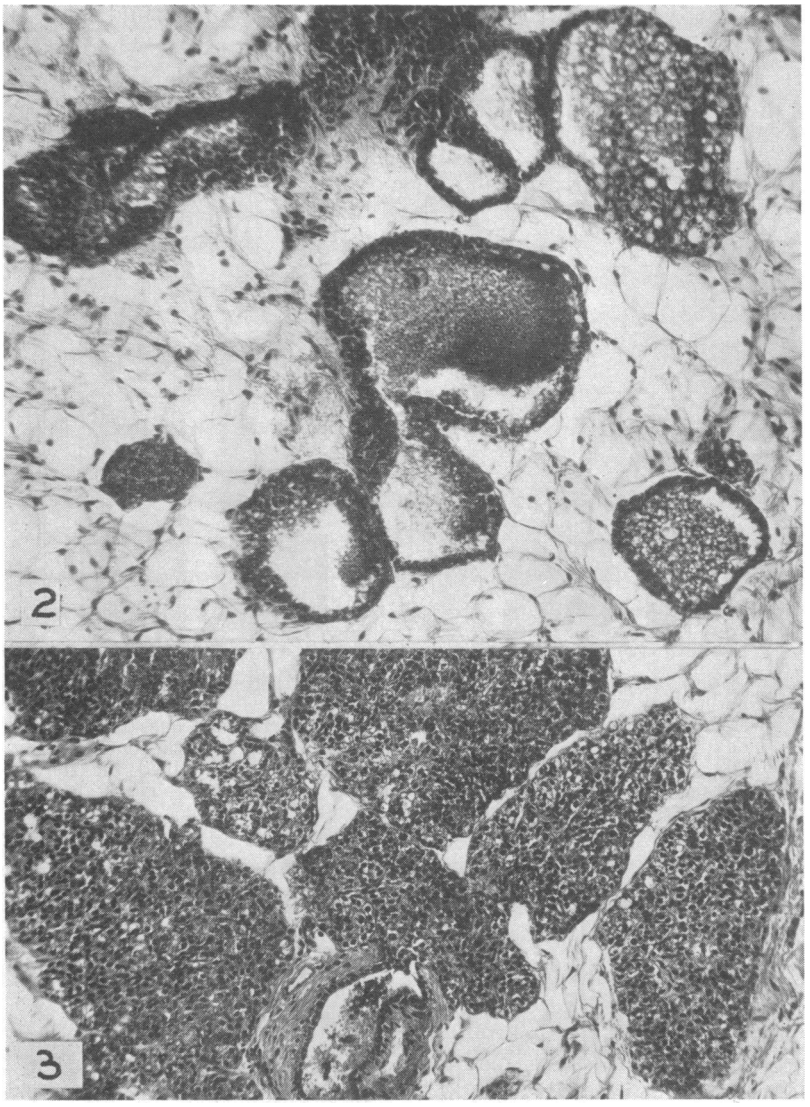


FIG. 1.—Alkaline phosphatase content of right inguinal mammary gland of rats which were fed 7,12-DMBA, 20 mg, at age 50 days and were treated with hormones for 9–29 days starting at age 65 days. The mammary glands were homogenized in 0.15 *M* NaCl containing 0.003 *M* $NaHCO_3$. Alkaline phosphatase was determined on the supernatant fluid following centrifugation at 11,000 *g*. The values are expressed in King-Armstrong[9] units per 1 mg of nitrogen.

The administration of estradiol-17β, 20 μg, stimulated alveolar development of lactational type (Fig. 2) within a short time and caused a twofold increase of alkaline phosphatase. The abundant secretion of milk within these glands undoubtedly results from increased lactogenic hormone secretion by the adenohypophysis. Progesterone, 4 mg, induced approximately a threefold rise of alkaline phosphatase of the mammary gland and a proliferation of the epithelium without secretion. The progressive increment in mammary alkaline phosphatase content in rats receiving progesterone for 29 days (Fig. 1) is due to the synergistic effect of endogenous phenolic estrogens, since progesterone alone has little effect on the alkaline phosphatase content of the mammary gland of ovariectomized rats.

The combined administration of estradiol-17β, 20 μg, and progesterone, 4 mg, induced extremely hyperplastic mammary glands (Fig. 3) with a fourfold increase of alkaline phosphatase. The proliferation was simply an exaggeration of the hyperplasia induced by progesterone alone. Secretion in the acini was minimal; there was no milk.

The administration of estradiol-17β, 20 μg, and progesterone, 4 mg, for a month depressed ovarian function. At the end of this time, in the hormone-injected rats, the weight of the ovaries was 20 $\pm$ 3 mg/100 gm of body weight; comparable ovarian weight of uninjected companion rats was 46 $\pm$ 6 mg/100 gm. But the function of the ovaries recovered remarkably and rapidly after cessation of these hormone



The photomicrographs are of paraffin sections stained with hematoxylin and eosin.

FIG. 2.—Mammary gland of rat which received estradiol-17 β, 20 μg, for 30 days, demonstrating hyperplasia with lactation. × 165.

FIG. 3.—Mammary gland of a rat which received estradiol-17 β, 20 μg, and progesterone, 4 mg, for 30 days, demonstrating hyperplasia without lactation. × 165.

injections: the first estrus occurred 6–9 days later, followed by regular 4-day cycle. Accordingly, the animals re-entered a normal endocrine status highly conducive to the growth of mammary cancer should it have been present, soon after stopping hormonal treatment.

Discussion.—These experiments depend on the experience that a single feeding of 7, 12-DMBA induces mammary tumors—rapidly and always under the conditions employed in the present work; in this laboratory there has been no failure to induce

mammary cancer in 600 consecutive normal female rats fed a single dose of 7, 12-DMBA, 20 mg, and untreated otherwise. The mammary tumors evoked are carcinomas (these occur early) and fibroadenomas which develop later than the cancers. To observe the development of the benign tumors, it is sometimes necessary to excise the cancers in order to preserve the life of the host.

The presence of mammary cancer was presumptive in the present experiments at the time the hormonal modifications were begun, namely 15 days after feeding the hydrocarbon. The cancers are not palpable at that time. But (1) we have found early mammary cancers upon microscopic examination of serial sections of the mammary glands at age 65 days, and (2) the cancers became palpable (8–10 mg in weight) shortly after age 70 days.

The hormonal modifications employed in the present work were for a limited duration—10 to 30 days only. After their cessation, the rats were untreated and observed for six months or longer. All of the hormonal changes induced proliferation in the normal mammary glands.

Pregnancy accelerated the development and growth of mammary cancers confirming an earlier observation;[7] the number of cancers of the breast was augmented. Progesterone in large amounts had a similar cancer-enhancing effect in intact female rats and non-lactational growth of mammary tubules was induced. Estradiol-17β merely postponed the appearance of mammary cancer; it did not extinguish the cancers, and the incidence of cancer of the breast eventually was 100 per cent of the rats. Estradiol-17β induced a moderate growth of mammary glands with the secretion of milk.

The combined administration of progesterone and estradiol-17β extinguished mammary cancer in 52 per cent of the rats; these animals remained free from cancer for six or more months. Moreover, in those rats which eventually developed mammary cancer, these hormones postponed the appearance and reduced the numbers of cancers. The mammary glands of rats receiving estradiol-17β concurrently with progesterone were extremely hyperplastic although without lactation; the large concentration of alkaline phosphatase in the mammary glands reflected their exuberant growth. The lactational effect caused by estradiol-17β was eliminated by simultaneous administration of progesterone.

Amounts of estradiol-17β with progesterone which were effective in suppressing mammary cancer caused a pronounced decrease of weight of the ovaries. But the ovarian depression was not long-lasting, since normal estrus cycles resumed 6–9 days following cessation of 30-day hormone treatment.

Mammary cancers were the only neoplasms which were extinguished by estradiol-17β with progesterone. Benign tumors of the breast and ear duct cancer developed in some of the rats in which the mammary cancer had been destroyed. It was found[8] in previous experiments that estradiol-17β given with progesterone accelerates the growth of benign tumors of the breast.

In sum, a considerable number of mammary cancers were extinguished by concurrent estradiol-17β and progesterone, whereas benign tumors of the breast and ear duct cancers were not eliminated and growth of normal mammary glands was promoted vigorously.

Earlier, it was discovered[10] that many of the mammary cancers in rat induced by polycyclic aromatic hydrocarbons have hormone-dependent qualities, since

established carcinomas regress after removal of ovarian steroids by means of ovariectomy or hypophysectomy. Hormone-dependent cancers differ in an essential manner from their normal cells of origin. Normal hormonal target cells survive and merely decrease in size and metabolic activity after removal of the appropriate hormones, whereas the hormone-dependent cancer cells die. Therefore, in cancers of this dependent class the hormones are of critical importance for the life of the cell. In the present experiments, a significant proportion of mammary cancers was destroyed, not by hormone withdrawal but by the administration of two hormones normally produced in the ovary; one of these (progesterone) given singly accelerated the cancerous process in intact rats, whereas the other (estradiol-17β) merely depressed the growth rate of mammary cancer. It would appear that the hormones in combination had interfered with that steroid hormone mechanism which is of cardinal, essential, and prime importance for the life of certain mammary cancers. Hormone-interference is a novel principle in extinction of cancer.

Extinguishment of mammary cancer by the administration of large doses of gonadotrophin shows that the ovary can be stimulated to secrete sufficient quantities of its own hormones to destroy cancers in the host.

Summary.—A single feeding of 7,12-dimethylbenz(a)anthracene to rats induced mammary cancer in all of the animals and benign mammary tumors in most of them. Hormone modifications which induced exuberant growth of normal mammary glands were furnished *for a limited time* in groups of animals which had been fed this hydrocarbon 15 days earlier.

Pregnancy accelerated the growth of mammary cancers and increased their numbers. Progesterone, alone, had a similar cancer-promoting effect in intact females. Estradiol-17β merely delayed the appearance of cancer of the breast, and eventually 100 per cent of the animals developed mammary cancer.

Estradiol-17β administered concurrently with progesterone destroyed a significant number of mammary cancers; 52 per cent of the rats treated with this combination of hormones apparently were free from cancer after six months. This is the extinction of cancer through hormone-interference with a steroid mechanism which is essential for the life of the malignant cell.

Estradiol-17β with progesterone induced extensive hyperplasia of normal mammary glands and did not destroy benign tumors of the breast or cancer of the ear duct. The combined administration of estradiol-17β and progesterone was not toxic, nor did it cause a long-continued depression of activity of the ovaries; normal ovarian function was restored promptly after cessation of 30-day hormone treatment.

* This work was supported by grants from the American Cancer Society and the Jane Coffin Childs Memorial Fund for Medical Research.

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