Antimammary Carcinogenic Activity of 17-Alpha-Ethinyl Estriol

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Both initiation and promotion of dimethylbenz(a)anthracene (DMBA)-induced mammary carcinogenesis were inhibited by prophylactic therapy for 1 to 7 months using 17-alpha-ethinyl-estriol in doses as low as 1.0 μg/d administered to intact virgin female Sprague-Dawley rats at 35 to 65 days of age. Administration of 638-μg single or multiple doses 2 to 3 weeks before DMBA induced a 75% to 85% reduction in cancer incidence after 1 year ($P < 0.001$). When treatment was begun 2 weeks after DMBA, 1.0 μg/d infused for 84 days resulted in a 44% reduction in incidence, with higher-dose, more prolonged therapy achieving a 73% reduction, equal to the reduction in carcinoma incidence observed after ovariectomy. Biopsies of nontumorous mammary glands showed a positive correlation between prelactational lobuloalveolar hyperplasia, hormone dose, and reduction in incidence of mammary carcinoma. Similar treatment with 17-alpha-ethinyl-estradiol-17B and diethylstilbestrol did not inhibit the 90% to 100% incidence of carcinoma observed in DMBA-treated control rats, and induced lactational hyperplasia in mammary gland biopsies. Continuous ethinyl estriol infusion subcutaneous (sc) in 2.5 to 7.5 μg daily dosage significantly increased uterine weights by as much as 10% to 46% after 2 to 4 weeks. At the time of mammary neoplasm development when rats were necropsied, no significant difference was observed in uterine weights between rats receiving 638 μg/mo in a readily soluble pellet implant, and uterine weights of control rats. Ethinyl estriol given seven times monthly in 638-μg bolus doses was more inhibitory of mammary carcinogenesis than estriol after a year ($P < 0.1 > 0.05$). Short-term intermittent administration of ethinyl estriol to young nulliparous women may offer a method of simulating the differentiating effect of pregnancy on mammary tissues, increasing durable resistance to carcinogenesis.

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ESTRIOL, estriol-3-methyl ether, and 6-keto-estradiol inhibited mammary carcinoma development after monthly implantation of 638 μg of crystalline estrogen sc in intact virgin female Sprague-Dawley rats fed 20 mg of 7, 12 dimethylbenz(a)anthracene (DMBA). The incidence of mammary carcinoma development in rats treated with estriol, estriol-3-methyl ether and 6-keto-estradiol incidence was less than that observed in control rats receiving only the carcinogen. Numerous other estrogens, including estrone, estradiol, ethinyl estradiol, diethylstilbestrol, and hexestrol, which were tested similarly, did not inhibit carcinogenesis.[1,2] Wotiz *et al.* confirmed the inhibitory activity of estriol against DMBA-induced mammary carcinogenesis and reported that continuous infusion of 1 μg of estriol every 24 hours reduced mammary carcinogenesis by about 50%.[3,4] Purdy *et al.* reported cell transformation in tissue culture induced by diethylstilbestrol, estrone, estradiol 17B; however, mouse fibroblast 3T3 cells were not transformed by estriol even in 50 μm concentration.[5-7]

Lan and Katzenellenbogen observed that 17-alpha-ethinyl-estriol was retained much longer than estriol in uterine nuclear estrogen acceptor sites, increasing the estrogen agonist activity.[8] Prophylactic ethinyl estriol therapy did not reduce mammary cancer incidence in DMBA-treated female rats when the initial dose was administered within 2 to 3 days of feeding DMBA.[2] However, induction of intense cell proliferation activity inhibited DNA repair in cells exposed to carcinogens.[9,10] As a result, we reinvestigated the antimammary carcinogenic activity of ethinyl estriol and other estrogens, using precautions not to immortalize unrepaired cell clones by estrogenic stimulation of the mammary glands during the period of maximal DNA damage by DMBA.[11-13] When ethinyl estriol treatment was initiated at least 2 weeks before or after DMBA feeding, ethinyl estriol was the most effective antagonist of mammary carcinogenesis we have identified thus far, during both initiation and promotion phases.

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TABLE 1. Inhibition of Mammary Carcinogenesis by Ethinyl Estriol and Other Therapy

Experiment no./treatment	Mammary carcinoma incidence (No. of rats with CA/total given DMBA)					
	Controls		Treated		Significance	
	210 d	365 d	210 d	365 d	U	χ^2
Treatment started 2 weeks before DMBA						
1 Ethinyl estriol 638 μg/mo × 7 at 35 d of age	8/10	9/10 (90%)	0/8	1/8 (13%)	<0.001	<0.001
2 Ethinyl estriol 638 μg at 50 d of age	7/11	7/11 (64%)	1/13	3/13 (23%)	<0.05	<0.025
3 Ethinyl estriol infused at 35 d						
2 μg/d × 28 d	11/12 (92%)	—	6/12	7/12 (58%)	<0.02	NS
6 μg/d × 28 d	11/12 (92%)	—	4/12	8/12 (67%)	<0.001	NS
Treatment started within 3 days of DMBA						
4 Ethinyl estriol	5/18	10/18	7/22	11/22	NS	NS
5 638 μg/2 mo × 7		(56%)		(50%)		
Treatment started 2 weeks after DMBA						
6 Ethinyl estriol 638 μg/mo × 19	9/12	10/12 (83%)	0/11	9/11 (82%)	<0.001	NS
7 Ethinyl estriol 638 μg/mo × 7	14/14 (100%)	—	2/15	4/15 (27%)	<0.001	<0.001
8 Ethinyl estriol infused 1 μg/d × 84 d	10/12	12/12 (100%)	4/9	5/9 (56%)	NS	<0.01
9 Ethinyl estriol 638 μg at 65 d	11/11 (100%)	—	6/12	9/12 (75%)	<0.05	NS
10 Estriol 638 μg/mo × 10	18/19 (95%)	—	5/19	12/19 (63%)	<0.001	<0.05
7 Ethinyl estradiol 638 μg/mo × 7	14/14 (100%)	—	9/10	10/10 (100%)	NS	NS
11 Diethylstilbestrol 638–320 μg/mo × 6	10/10 (100%)	—	7/8	8/8 (100%)	NS	NS
Castration 2 weeks after DMBA						
12, 13	21/30	26/30 (87%)	2/19	5/19 (26%)	<0.001	<0.001

DMBA: dimethylbenz(a)anthracene; NS: not significant; CA: carcinoma; χ^2: chi-square test; U: Mann–Whitney U test.

Materials and Methods

Virgin female Sprague-Dawley albino rats derived from a Holtzman strain (Sasco, Omaha, NE) were obtained at 35 to 50 days of age. They were randomized on receipt into individual suspended cages, fed Purina Lab Chow pellets (Purina, St. Louis, MO) *ad lib.* and given distilled water to drink. The cage room had automatic timing of lights providing a 12 hour light-dark cycle. The temperature was maintained between 22.2 and 24.4°C and the relative humidity between 30% to 50%. The carcinogen, DMBA (Eastman Kodak, Rochester, NY) was dissolved in sesame oil immediately before administration, in a concentration of 20 mg/1.5 ml of oil for each dose. This was given under light ether anesthesia using a flexible 12-cm gavage tube; all operative procedures were performed similarly, and rats to be killed because of neoplasms were anesthetized before cervical fracture. Rats were weighed and examined every 10 to 14 days for neoplasms. The location of each neoplasm and its size in two diameters were recorded before the rat was killed. Frequently one or more of the normal-appearing mammary glands were excised for histologic examination along with mammary tumors, fixed in 10% formalin, and stained with hematoxylin and eosin. All slides were read double blind.

Hormonal treatment of 50% to 66% of each batch of age-matched rats was begun either 14 to 21 days before DMBA therapy, or 13 to 14 days following. Two methods were used, with the most commonly used method monthly subcutaneous implantation of pellets 1 mm in diameter, 1 to 2 mm in length, weighing 6.38 ± 0.175 SD mg. These contained 10% of the hormone to be administered in crystalline NaCl (w/w), (unless oth-

erwise specified) and were manufactured in a Forbes manual pellet press.[1] The second method was sc implantation of Alzet osmotic minipumps (Alza Corporation, Palo Alto, CA), with the designated hormone dose contained in a vehicle composed of 5% ethyl alcohol (ETOH), 25% distilled water, and 70% propylene glycol. Ethinyl estriol was obtained from Eli Lilly, (Greenfield, IN) and other estrogens either from Sigma Chemical, (St. Louis, MO) or Steraloids (Wilton, NH).

The extent of reduction of cancer incidence in treated rats was compared with that in untreated DMBA-fed controls after 7 months and 1 year and evaluated for significance by the chi-square test or the Fisher exact probability test. The increase in the number of mammary carcinoma-free rat–days in the treated group compared with that observed in the controls was tested for significance by the Mann–Whitney U test of rank-ordered individual rat–days of survival.

At the time of necropsy, the uterus was dissected, and wet and dry weights were obtained. The student's *t* test was used to determine significance of mean differences in uterine weights for a two-tailed distribution. Rats who did not develop neoplasms were observed for at least 1 year, before autopsy. All rats who died during the course of an experiment were necropsied to ascertain the cause of death and to determine whether any neoplasm was present that might have escaped notice at the routine biweekly examination.

The effect of endocrine treatment upon ovulation was determined by administering a 638 μg dose at the time of proestrus, and then seeing whether eggs could be identified 24 hours later in the Fallopian tubes using a dissecting microscope. Estrus cycles were evaluated from antemeridium (AM) vaginal cytology in selected experiments.

Nontumorous mammary gland biopsies were scaled on a + − ++++ basis for alveolar hyperplasia, and separately scored for evidence of lactation.

Results

Reduction of Mammary Carcinogenesis Incidence by Ethinyl Estriol

As shown in Table 1, ethinyl estriol therapy induced a highly significant reduction in mammary carcinoma incidence after 7 months' observation when treatment was begun 2 weeks before initiation of carcinogenesis, or two weeks afterwards, but not when the hormone and DMBA were given within a few days of each other. Only 11 of 45 (24%) of rats treated early developed neoplasms at 7 months *versus* 37 of 45 (82%) of controls, and the reduction in incidence was sustained for at least a year. All experiments led to a significant increase in mammary cancer-free survival by the Mann-Whitney U test.

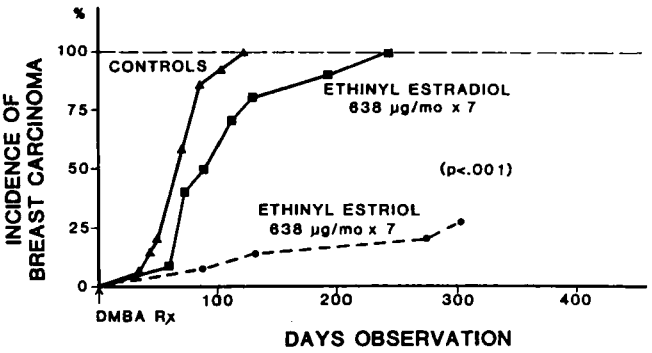


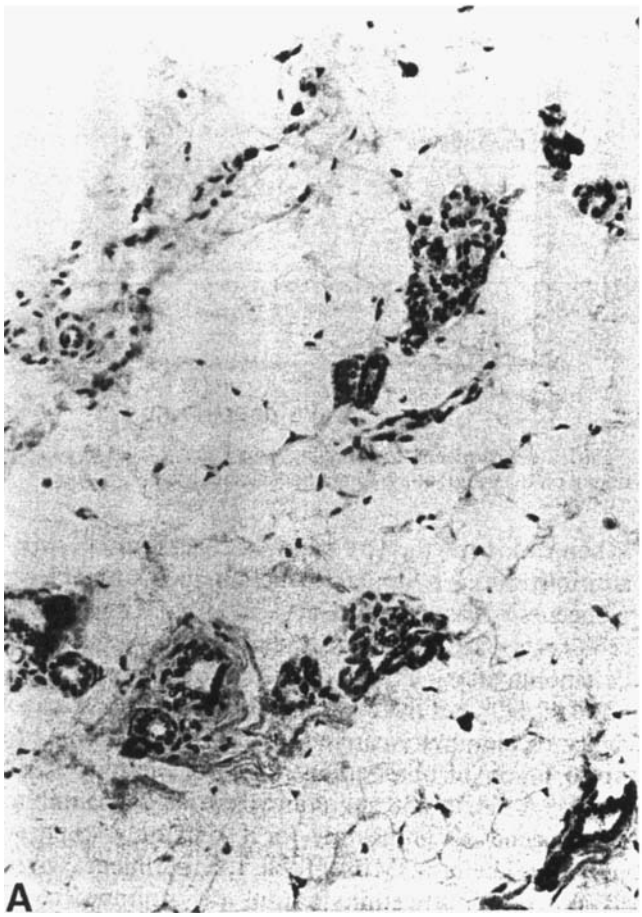
FIG. 1. Comparison of 17a-ethinyl-estriol and 17a-ethinyl-17B-estradiol on the promotion of mammary carcinogenesis.

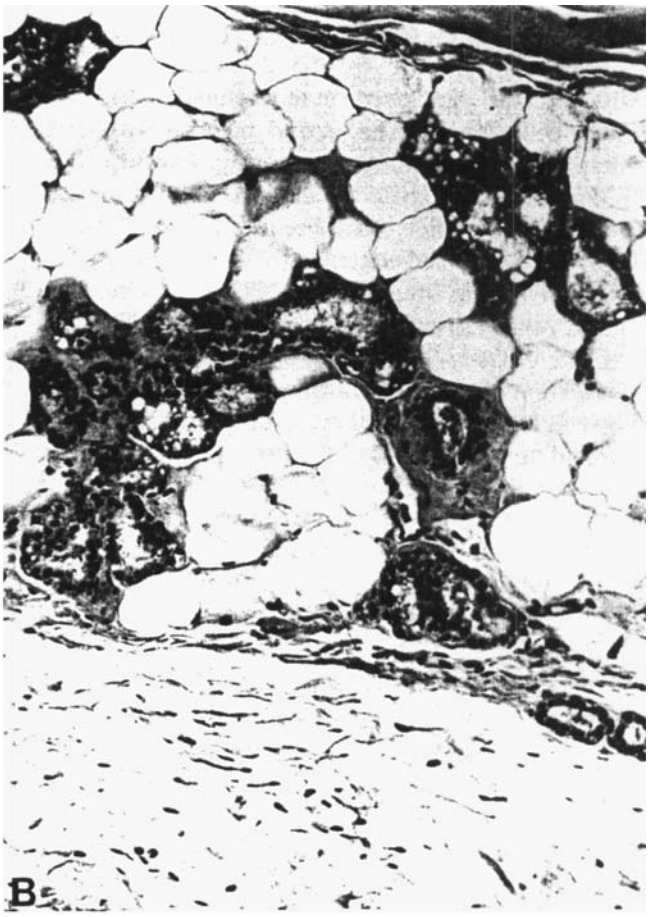
When endocrine therapy followed 2 weeks after DMBA administration, a similar reduction in mammary carcinogenesis was achieved at 7 months. Twelve of 47 (26%) rats with delayed treatment developed mammary carcinoma after 7 months' observation, compared with 44 of 49 (90%) of the controls. These reductions of incidence of mammary carcinoma also were sustained for up to 1 year of observation in two of the four experiments. A single 638 mg ethinyl estriol dose inhibited carcinogenesis significantly for 1 year only when administered before DMBA (Table 1, Experiment 2 *versus* 9). When monthly ethinyl estriol was continued indefinitely, there was increasing likelihood of induction of lactation, and late development of mammary neoplasms (Table 1, Experiment 6). The minimum effective prophylactic dose of ethinyl estriol was similar to that reported for estriol, 1.0 μg every 24 hours, administered for 3 months.[4] Doubling this dose for 28 days resulted in a similar reduction of cancer incidence (Table 1, Experiment 3).

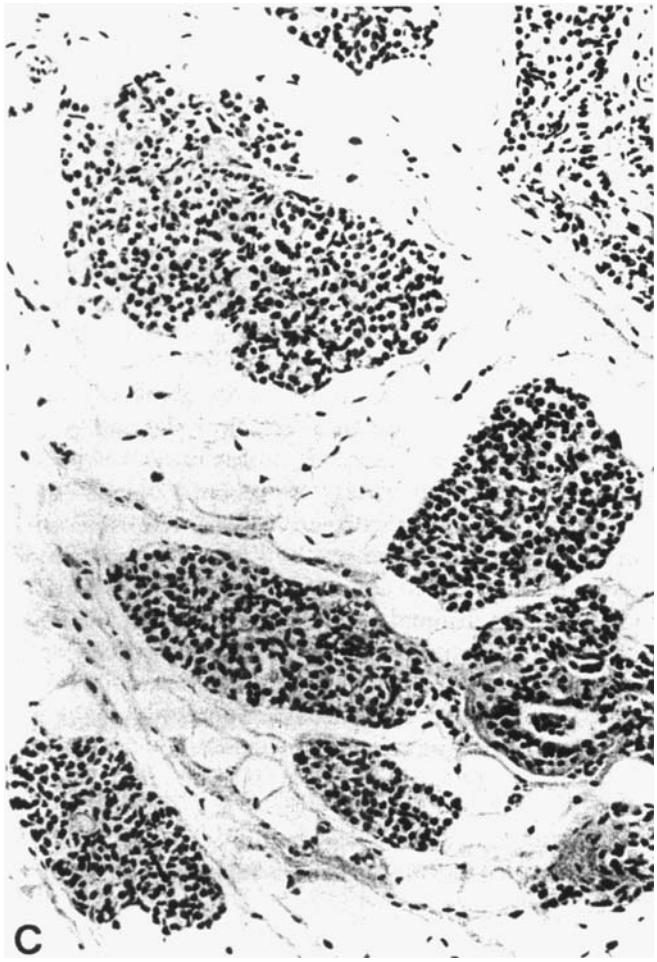
The maximum reduction in cancer incidence achieved by ethinyl estriol therapy after 1 year was about 25% or less than the corresponding control incidence (Table 1, Experiments 1, 2, and 7), which equalled the results of surgical castration peformed two weeks after DMBA induction of cancer (Table 1, Experiments 12 and 13). Mammary carcinogenesis was not inhibited by the administration of similar dosages of 17-alpha-ethinyl-estradiol, or diethylstilbestrol (which required dose reduction because of excessive inhibition of growth and overstimulation of the uterus) (Table 1, Experiments 7 and 11; Fig. 1). Ethinyl estriol appeared about twice as inhibitory of mammary carcinogenesis as estriol given in identical dose ($P < 0.1 > .05$) (Table 1, Experiment 7 *versus* 10); however, the two hormones have not been directly compared in the same experiment.

Induction of Mammary Hyperplasia by Treatment

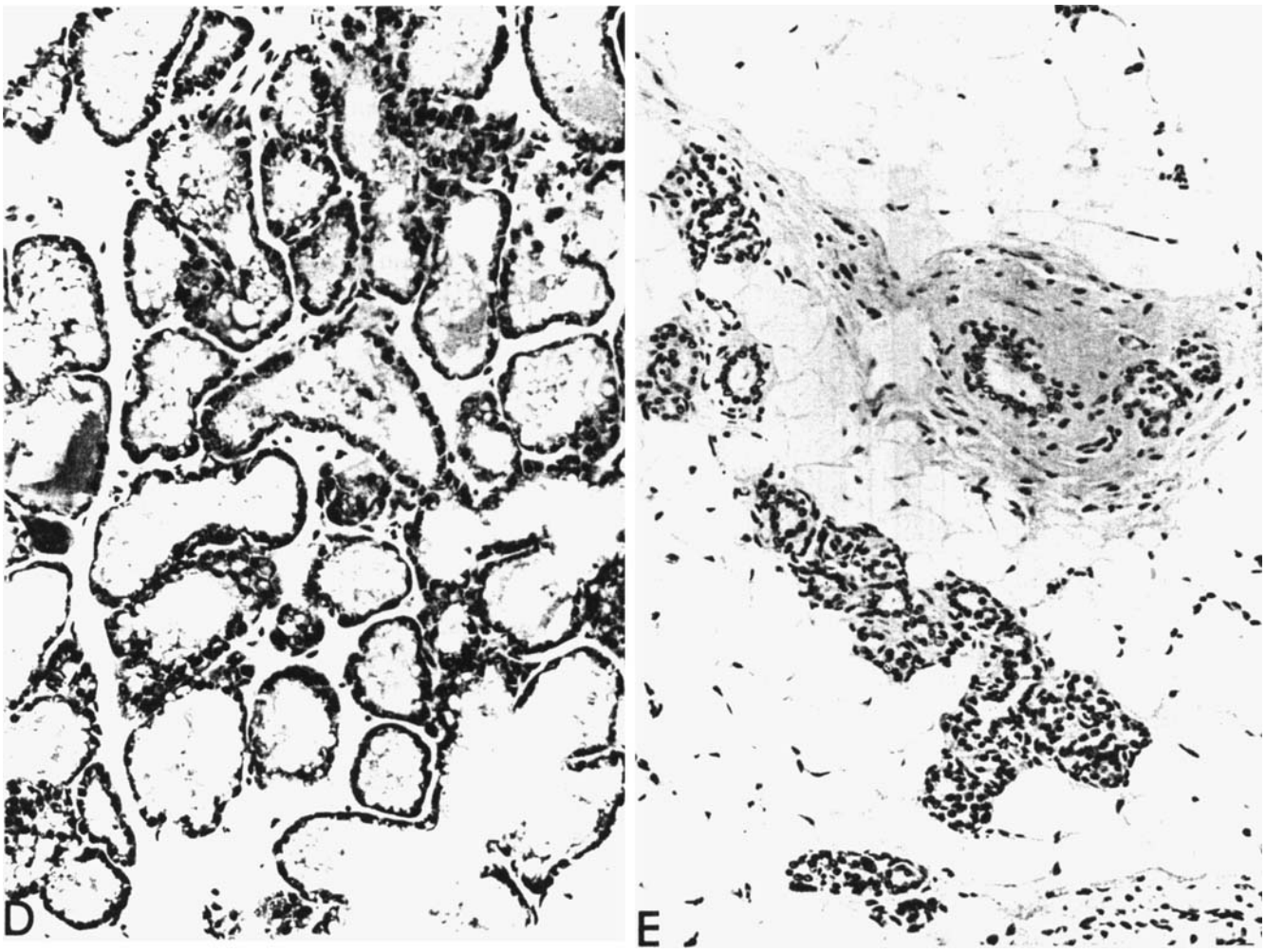
Mammary gland biopsies of ethinyl estriol-treated rats revealed alveolar hyperplasia in lobules. The degree







FIGS. 2A–2E. (Figs. 2D and 2E on facing page) Mammary differentiation induced by infusions of 17a ethinyl estriol and 17a ethinyl 17B estradiol. Virgin 50-day-old female rats unexposed to carcinogen were continuously infused sc with 2 μg/d or 6 μg/d of estrogen. After 28 days they were killed; and the axillary mammary fat pads were biopsied and fixed in 10% formalin. Sections cut from paraffin blocks were stained with hematoxylin and eosin (×430). (A) 80 day old untreated gland. (B) Ethinyl estradiol 2 μg/d; Note lactation. (C) Ethinyl estriol 2 μg/d; intense lobulo-alveolar proliferation without lactation. (D) Ethinyl estradiol 6 μg/d; all acini distended with secretion. (E) Ethinyl estriol 6 μg/d; acini without lipid-containing secretion.



FIGS. 2D AND 2E.

of alveolar hyperplasia was proportional to dosage. After 1 μg infusion for 84 days, little or no change was detected from biopsies of control rats of similar age. Definite hyperplasia appeared after 2 μg had been infused for 28 days; higher doses showed even more marked differentiation of the virginal rat mammary glands (Figs. 2A, 2C, and 2E). After two 638-μg monthly ethinyl estriol doses, 50% replacement of periductular adipose tissue by alveolar hyperplasia occurred. No lactation was detected during the first 6 months of treatment, either as cytoplasmic vacuolization or as lipid-containing secretions in the ducts. After 6 months, gross and microscopic evidence of lactation among females still treated with monthly ethinyl estriol pellets increased.

In rats infused with ethinyl estradiol for as short a time as 2 weeks, cytoplasmic vacuolization and duct distention with secretions consistent with a hyperprolactinemic response occurred (Figs. 2B and 2D).

Response of the Genital Tract to Estrogen Therapy

When necropsy was performed on mammary tumor-bearing rats, no significant uterine hypertrophy was found among those treated with a single 638 μg ethinyl estriol pellet at 35 to 65 days of age, nor in those commencing treatment at approximately 65 days of age with monthly 638-μg bolus doses for 7 months (Fig. 3). In one experiment, a significant reduction in wet uterine weight occurred in treated rats compared to controls, which previously was observed after similar treatment with estriol.[2]

After continuous ethinyl estriol infusion of 1.0 to 7.5 μg per 24 hours for 2 to 4 weeks, both uterine wet and dry weights increased significantly (Fig. 3). Infusions of ethinyl estradiol induced a somewhat greater degree of uterine hypertrophy at each dose level than observed with infusions of ethinyl estriol. Uterine hypertrophy

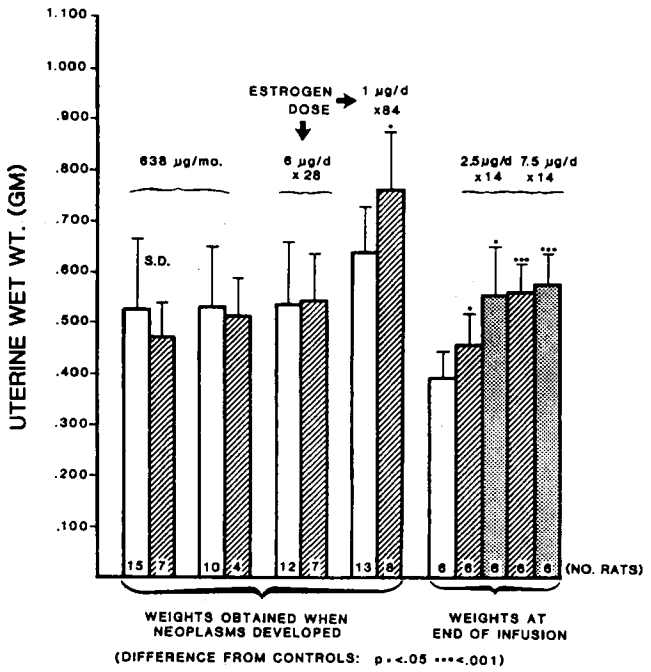


FIG. 3. Uterine wet weights after estrogenic therapy. □: Controls; ▨: 17-α-ethinyl-estriol; ⊠: 17-α-ethinyl-estradiol.

persisted long after termination of infusion therapy with 1.0 µg/24 hours of ethinyl estriol for 84 days (starting at 65 days of age).

Implantation of 638 µg of ethinyl estriol during proestrus was followed by ovulation within 24 hours in two female rats; similar treatment with ethinyl estradiol inhibited ovulation. Continuous infusion of ethinyl estradiol suppressed estrus cycles to a greater degree than ethinyl estriol infusions of similar dose (Table 2). After completion of 7 months of ethinyl estriol 638 µg/mo, regular estrus cycles continued in most rats. No proliferative or cystic ovarian lesions were noted at necropsy

TABLE 2. Estrus Cycles Noted During a 21-Day Period of Estrogen Infusion (Exp. 14)

Treatment	N	No. of cycles/21 d.	
Ethinyl estradiol infused continuously			
2.0 µg/d	4	0	
2.0 µg/d	4	2	
6.0 µg/d	4	0	mean 1/rat
6.0 µg/d	4	2	
Ethinyl estriol infused continuously			
2.0 µg/d	4	1	
2.0 µg/d	4	3	mean 2.5/rat
6.0 µg/d	4	2	
6.0 µg/d	4	4	
Control—no infusion	4	4	mean 5/rat
Control—no infusion	4	6	

in ethinyl estriol treated rats, but several uteri distended with intrauterine hemorrhage were noted in rats receiving more than 7 months of bolus monthly therapy. Ethinyl estriol treatment reduced somatic growth rate by 4% to 12% in the first 4 months of observation.

Discussion

In this investigation, estrogen-induced mammary epithelial proliferation was avoided during the most acute phase of molecular disruption of DNA resulting from the administration of DMBA.[12,13] As a result, it has been possible to show that ethinyl estriol markedly inhibited the development of mammary carcinomas either when given before or after cell transformation by the carcinogen in the majority of treated rats for at least 1 year (Table 1). Ethinyl estriol previously did not affect the incidence of mammary carcinoma when it was administered within 2 to 3 days of feeding DMBA[2] (Table 1, Experiments 4 and 5).

The prophylactic effect of 7 months of intermittent ethinyl estriol treatment initiated 2 weeks after DMBA was equivalent to castration, after 1 year of observations, suggesting that inhibition of estrogen-dependent cancer promotion was involved. Prolonged endocrine therapy was unnecessary, since only enough ethinyl estriol was needed to induce some degree of nonlactational lobuloalveolar epithelial hyperplasia in treated rats to confer protection from carcinogenesis for the duration of the observation period; a positive correlation was demonstrated between hormone dose, degree of nonlactational alveolar hyperplasia, and reduction in subsequent mammary carcinogenesis following DMBA therapy. Ethinyl estradiol and diethylstilbestrol in similar dosage did not reduce mammary carcinogenesis, although mammary alveolar hyperplasia was induced as well, accompanied by histologic evidence of lactation. Intermittent monthly bolus doses of ethinyl estriol continued past 7 months resulted in increasing gross and microscopic lactation and increased neoplasia (Table 1, Experiment 6).

Modification of the incidence of DMBA-induced rat mammary carcinogenesis by estrogen and other endocrine therapy has recently been reviewed.[14] The majority of reported investigations have not followed the treated female rats for longer than 6 months, which does not fully determine the durability of inhibition of promotion of carcinogenesis. Kledzik *et al.* reported complete suppression of mammary neoplasia of up to 155 days' duration by administering 20 µg/d of estradiol for 20 days before and after intravenous DMBA, alone or with 4 mg of progesterone.[15] Only 67% of estrogen-treated rats survived, with more than a 33% reduction in growth rate compared with controls. Grubbs *et al.* repeated this

experiment in N-nitroso-N-methylurea-induced mammary cancers without complications, observing a 79% reduction of mammary carcinoma incidence from that seen in controls after 6 months' observation.[16] The carcinogen was administered following completion of the hormonal therapy in this investigation. Kelly *et al.* followed DMBA-treated rats for only 130 days in their observation of a dose-related inhibition of mammary carcinogenesis by 11-alpha-methoxy-17-alpha-ethinyl-estradiol-17B.[17] Doses of 0.5, 2.0, 8.0, and 24 μg daily were administered beginning on the day DMBA was given, resulting in 22%, 60%, and 100% inhibition of tumor promotion, respectively. The 2.0 μg/d dose reduced unoccupied estradiol, progesterone, and prolactin cytosol receptors in mammary neoplasms, but doses as high as 8.0 μg did not alter plasma prolactin activity. Kellen reported that pregnancy initiated 5 days before or after DMBA administration completely suppressed the appearance of mammary neoplasms for as long as 200 days.[18] Biweekly chlorotrianisene 5 mg, a synthetic estrogen administered for 10 weeks also suppressed tumorigenesis.[18] Dao confirmed the inhibitory effect of pregnancy upon mammary carcinogenesis if induced within 25 days of DMBA treatment.[19]

We have confirmed the mammary cancer inhibitory activity of the more estrogenic B epimer of 11-methoxy-17a-ethinyl-estradiol using our treatment protocol, delaying the initial dose for 2 weeks after DMBA.[20] Therefore, therapy with both natural and synthetic steroidal estrogens as well as nonsteroidal estrogens administered before, during, and after the period of maximum mammary epithelial transformation by DMBA can induce a marked delay in tumor promotion, as well as appropriately timed conception and pregnancy. Inhibition of mammary cancer promotion probably requires initially a combination of local mammary epithelial differentiation by the estrogen, altered state of the mammary estrogen receptors and minimal augmentation of hypophyseal prolactin-growth hormone secretion.[17]

Increasing experimental evidence supports the concept that hormonal induction of differentiation in the virginal rat mammary gland inhibits both initiation and promotion of carcinogenesis.[13,16,21-24] This concept also has been extended to the differentiation achieved by pregnancy in the human mammary gland.[25]

During pregnancy, there is about a 1000-fold increase in estriol production by the feto-placental unit, which rapidly rises towards term, when 25 to 50 mg of conjugated estriol is excreted in the urine.[26] Fetal plasma total estriol concentrations range several-fold higher than maternal during the last trimester.[26] Total plasma estriol concentrations reach 9 to 25 μg/dl in the maternal circulation at term, several times higher than total estradiol, which is more highly protein-bound to carrier proteins, and hence probably with less biologic activity.[27] The net result is marked uterine hyperplasia, and mammary hyperplasia and differentiation in preparation for lactation.

Initial pregnancy reduces or increases risk of breast cancer compared with that encountered by nulliparous women, depending on the age of the woman at childbirth. Pregnancy within 5 years of onset of menstrual function reduced risk of nonfamilial breast cancer by over 50% before the widespread introduction and use of contraceptive estrogens.[28,29] Delay in initial conception to the age of 25 to 30 years removed any benefit, and later initial childbirth increased breast cancer risk up to 40% above that of nulliparae. The protective effect of multiple pregnancies reported in many prior investigations was attributed largely to the earlier age of initial pregnancy of many multiparous women, but in more recent prospective investigations, a progressive decrease in cancer risk has been demonstrated for up to four pregnancies.[30,31] Basal prolactin plasma concentration of nulliparous women was found to be 25% to 35% higher than parous siblings.[32] Initial pregnancy, regardless of the age of conception, resulted in a durable 50% reduction in basal and perphenazine-stimulated plasma prolactin concentration after delivery.[33] There was also a 14% to 25% increase in serum estriol concentration (without any change in other serum estrogens) following pregnancy lasting for many years.[34] Serum dehydroepiandrosterone and its sulfate ester fell significantly after pregnancy, suggesting that the increased estriol was largely derived from increased aromatase activity. Additional pregnancies did not induce further changes in hormonal metabolism. In addition, between the ages of 15 and 28 years, a marked negative correlation has been reported between age of first pregnancy, and the rate of conversion of estrone to estriol, which is the principal virginal pathway for estriol biosynthesis.[35] The fall of prolactin activity and the increased plasma ratio of estriol to estradiol in parous women tend to reduce breast cancer risk.

Plasma prolactin increases about eightfold above basal nonpregnant values by the 30th week of pregnancy, accompanied by the much larger increased production of total estrogens by the fetoplacental unit.[36] This maternal endocrine milieu would promote hormone-dependent asymptomatic microcancers that may appear in the breast within a few years of menarche and increase in number with age, so that up to 25% of women may become diseased.[37] The age-related increase in the microcancers may account for the waning prophylactic benefit of initial pregnancies conceived in older women.

Continuous daily infusion of as little as 1.0 to 2.0 μg of unconjugated ethinyl estriol for only 1 to 3 months sig-

nificantly reduced DMBA-induced mammary carcinoma incidence (5 to 10 μg/kg/d), a minimum cancer inhibitory dosage previously reported for estriol.[4] If only 1% to 2% of pregnancy-secreted estriol were not conjugated, or estriol-3-sulfate biologically active as seems likely, the estimated 10 μg/kg/24 hour necessary to induce prelactational lobuloalveolar hyperplasia and differentiation of the virgin human mammary gland would be exceeded. If this differentiation is induced sufficiently early after menarche, before the carcinogenic activity of estradiol has transformed significant numbers of epithelial cells,[6,7] optimal reduction of breast cancer risk may be achieved.[28,29] This investigation therefore extends previous reports concerning the "estriol hypothesis" relative to the inhibitory role of early pregnancy in modifying breast cancer incidence.[1-4,38,39]

The uterine response to estriol or ethinyl estriol would be important in considering prophylactic therapy. Some uterine hypertrophy developed even at the lowest continuous dose of 1.0 μg daily, after several months (Fig. 3). Much larger monthly bolus doses of ethinyl estriol did not induce uterine hypertrophy compared with controls even after 7 or more months of treatment, probably because of rapid absorption and excretion within 2 weeks.[2] This treatment protocol moreover gave the best inhibition of carcinogenesis, equivalent to surgical castration (Table 1). Therefore, intermittent prophylactic estriol treatment would seem least likely to have uterine side effects.

Confirmation and extension of these observations defining especially the minimum dose needed and duration of continuous or intermittent treatment in primates is necessary before consideration might be given to Phase I human trials in nulliparous young women for potential mammary cancer prophylaxis. The prolonged intense estriol exposure of all fetal and maternal tissues before birth is not consistent with any appreciable hazard from carcinogenesis induction by estriol therapy of young nulliparous women, to induce prelactational mammary gland differentiation. Experimental induction of cancer in rodents by very large estriol concentrations administered for many months has been reported infrequently[40,41] and has lacked pathologic confirmation.[42] In the kidneys of castrated male hamsters, 17-alpha-ethinylation of estradiol decreased carcinogenic activity, and estriol was minimally carcinogenic,[43-45] in keeping with its minimal cell transforming activity *in vitro*.[6,7] No major effort has yet been made for primary prophylaxis of mammary carcinoma in the US, where the crude annual incidence rate is slowly rising to 85 per 100,000 persons, with over 40,000 annual deaths.[46] If the physiologic maturation of the human mammary gland characteristic of pregnancy can be simulated by estriol or ethinyl estriol therapy without feto-placental function, before estradiol-induced epithelial cell transformation has occurred, a rational basis for such an effort may exist.

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