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Neoplastic Responses and Correlated Plasma Prolactin Levels in Diethylstilbestrol-treated ACI and Sprague-Dawley Rats[1]

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ABSTRACT

Pellets containing 5 mg diethylstilbestrol (DES) and 15 mg cholesterol were implanted s.c. in 84-day-old female Sprague-Dawley (S-D) and ACI rats. [*G*-^3H]DES (32.9 nCi/mg of DES) was incorporated into each pellet. Animals were decapitated, blood was collected at 0, 2, 10, 28, 56, 130, and 214 days after implantation, and the amount of [*G*-^3H]DES remaining in each pellet was determined. Plasma prolactin levels were determined by radioimmunoassay.

DES was released from the implanted pellets exponentially, and the release was not significantly different in S-D rats than in ACI rats. No mammary tumors developed in any treated or untreated S-D rats. In contrast, 90% of the DES-treated ACI rats had developed two or more mammary adenocarcinomas by 130 days, and by 214 days 90% had four or more mammary adenocarcinomas.

A significant increase in the weight of the pituitary was noted in DES-treated ACI rats by 28 days. By Day 130, the pituitaries of the treated ACI rats were 2 to 7 times as heavy as were controls, and plasma prolactin levels were 10 to 40 times higher than in controls. In contrast, the pituitaries of treated S-D rats did not significantly increase in weight, and plasma prolactin levels were only 3 to 5 times higher than controls. As early as Day 10, the uteri of treated S-D rats were significantly heavier than those of control rats and contained large amounts of fluid. This effect was not seen in ACI rats. Although the release of DES from the implanted pellet was essentially the same in ACI and S-D rats, three distinctive strain differences in response to DES were noted: mammary adenocarcinomas were found only in treated ACI rats; pituitary prolactin-cell adenomas and associated elevated plasma prolactins levels were seen only in treated ACI rats; and pyometritis was induced only in treated S-D rats. Mammary adenocarcinomas and prolactin-cell adenoma responses in the treated ACI rats appear to be correlated with the increasing levels of plasma prolactin. This study demonstrates that the prolonged estrogen treatment of ACI and S-D female rats produces distinctly different mammary and pituitary neoplastic responses. This disparity in neoplastic responses appears to be reflected in the difference of degree to which the hypophysial prolactin cells are stimulated to grow and secrete hormone.

INTRODUCTION

Prolonged estrogen treatment can induce mammary tumors in certain strains of rats but not in others (7, 8, 10). The mechanisms responsible for these strain differences are not known but are thought to involve prolactin secretion. It is widely accepted that estrogens are the primary stimulators of prolactin secretion (22) and that prolactin has the primary role in the development and growth of mammary tumors (16, 22, 34). However, definite correlations between prolactin levels and estrogen-induced mammary tumors have not been reported.

The present comparative study was designed to investigate the relationships and possible correlations between estrogen treatment, prolactin levels, mammary development, and the induction of neoplasia in the mammary tissue and the pituitary glands of 2 rat strains, ACI and S-D.[4] Female rats of these 2 strains have distinctly different sensitivities to physical (26, 28) and chemical carcinogens.[5] The present experiment was part of an investigation of the hormonal mechanisms involved in the reported strain specific mammary neoplastic responses to DES treatment in combination with X-ray (26) or neutron irradiation (27, 28).

MATERIALS AND METHODS

One hundred female ACI (A × C, Irish) rats (Microbiological Associates, Inc., Bethesda, Md.) were obtained when they were 29 days old, and 100 female S-D rats (Sprague-Dawley Inc., Madison, Wis.) were obtained when they were 22 days old. All rats were maintained on commercial rat chow and water *ad libitum*, with 12 hr (8 a.m. to 8 p.m.) of fluorescent light per day, at 22.5 ± 1° and 55 ± 5% humidity.

At 84 days of age, 60 ACI and 60 S-D rats each received a compressed pellet containing 5 mg DES and 15 mg cholesterol. Thirty ACI and 30 S-D female rats served as untreated controls. Ten ACI and 10 S-D control rats each received a compressed pellet (20 mg) containing only cholesterol. These pellets were implanted s.c. in the intrascapular region of each rat with light ether anesthesia.

Preparation of DES Pellets. Pellets were prepared by adding approximately 50 μCi of [*G*-^3H]DES (TRK-50, 11 Ci/mmol; Amersham/Searle Corp., Arlington Heights, Ill.) to 1.5 g of DES and 4.5 g of cholesterol (Steraloids, Pawling, N. Y.) dissolved in 40 ml of toluene. After mixing, the solution was heated to 60° and evaporated to dryness under a stream of dry nitrogen. The final traces of the solvent were removed under a vacuum (20 torr). The dry mixture was used to prepare compressed pellets (20 ± 1.5 mg; 3.2 mm in diameter x 2.5 mm in length) in a manual pellet press (Parr Instrument Co., Moline, Ill.). The pellets contained

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[4] The abbreviations used are: S-D, Sprague-Dawley; DES, diethylstilbestrol.

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32.9 nCi [G-^3H]DES/mg of DES when assayed.

Determination of DES Released from Pellet. Following its removal from the animal, each pellet was placed in a 10-ml volumetric flask and crushed with a stirring rod; then the connective tissue capsule around the pellet was disassociated by shaking in 1 ml of hot (60°) 0.01 N HCl for 10 min. Two ml of methanol were added, and the solution was evaporated to dryness in a 60° water bath aided by a stream of dry nitrogen gas. The residue was dissolved in toluene, and the volume was brought up to 10 ml. A 1-ml aliquot was removed and placed in a scintillation vial, and 10 ml of scintillation counting solution (4 g PPO plus 50 mg POPOP per liter of toluene) were added. Samples were counted to 0.3% counting error in a Beckman Model LS-233 liquid scintillation counter (average ^3H efficiency, 58.3%). Quenching was checked by both the internal standard and the external standard methods and was found to be less than 1%. Therefore, a quench correction was not applied to these data. The amount of DES released from each compressed pellet was determined as follows:

$$X = \frac{B - A}{B} \times C = \frac{C(B - A)}{B}$$

$$B = C \times \frac{32.9\ \text{nCi}}{\text{mg DES}} \times \frac{2.22 \cdot 10^3 \text{dpm}}{\text{nCi}}$$

In which X is mg of DES released from pellet; A is dpm of [^3H]DES in pellet, after implantation period; B is dpm of [^3H]DES in pellet, prior to implantation; and C is mg DES in pellet = pellet weight prior to implantation (mg)/4.

General Protocol. Groups of animals were killed by decapitation at 0, 2, 10, 28, 56, 130, and 214 days after pellet implantation as shown in Table 1. Each rat was identified by a numbered ear tag. The rats were palpated once a week, and the anatomical location of each mammary tumor was recorded using the nipples as reference points. At autopsy, each animal was examined for gross pathological changes, and each pellet was carefully removed and assayed for DES. Each quandrant of breast tissue was removed, fixed, defatted, stained with hematoxylin, cleared in methyl salicylate, and examined at ×10 with a dissecting microscope. The mammary tissue development of each quadrant was evaluated and was given a rating of 1 to 5 to the nearest 0.5, where 1 to 3 indicated progressive degrees of duct growth and development, and 4 to 5 indicated lobuloalveolar growth (32). All mammary tumors were fixed, sectioned, stained with hematoxylin and eosin, and then given a pathological classification according to the criteria of Young and Hallowes (35). Abnormal pituitary glands were classified as gross tumors if they were hemorrhagic, fragile, or exceeded 30 mg in weight (5). Pituitary gland sections were examined immunohistochemically to determine the primary cell type present in the neoplasm (14).

Radioimmunoassay for Prolactin. Rats were killed quickly by decapitation, and the blood was collected in heparinized (80 μg/ml blood) centrifuge tubes. Plasma samples were separated by centrifugation at 1000 × *g* for 45 min at 4°, and then stored at −25° until assayed. All plasma samples were assayed at 2 dilutions in triplicate. The rat prolactin reference standard (RP-1), rat prolactin for radioiodination (I-1), and antiserum to rat prolactin (S-2) were obtained from the National Institute of Arthritis, Metabolism, and Digestive Diseases. The radioimmunoassay procedure used was a modification of the National Institute

Table 1

Responses of female ACI and S-D rats to DES pellet implantation

Group	DES released: Total (μg) ACI	Total (μg) S-D	Rate[a] (μg/day) ACI	Rate[a] (μg/day) S-D	Mean plasma prolactin (ng/ml) ACI	ACI	S-D	S-D	Mean mammary development[b] ACI	S-D	Rats with mammary tumors[c] ACI	S-D	Mean pituitary wt (mg) ACI	S-D
Day 0 control					39	(7-200)[d]	11	(5-38)	2.0	1.5	0/10	0/10	12.7	12.2
Day 2 DES	170	230	38	38	380[e]	(154-630)	39[f]	(10-131)	2.0	2.0	0/10	0/10	9.8	13.3
Day 10 DES	345	321	16	15	412[e]	(298-973)	68[e]	(42-11)	4.0	4.0	0/10	0/10	12.9	12.1
Day 10 control					14	(12-17)	20	(7-37)	2.0	2.0	0/5	0/5	10.7	14.4
Day 28 DES	495	453	8.9	7.9	414[e]	(282-833)	136[e]	(75-330)	3.5	3.5	0/9	0/10	19.3[f]	18.8
Day 28 control					17	(5-25)	14	(10-18)	2.0	2.0	0/5	0/5	12.5	17.0
Day 56 DES	743	588	6.1	5.3	1297[e]	(845-1700)	100[e]	(65-140)	3.5	3.0	0/10	0/8	28.1[e]	16.0
Day 56 control					57	(7-200)	13	(8-20)	1.5	2.5	0/5	0/5	14.8	16.7
Day 130 DES	1180	1220	3.9	3.2	1399[e]	(410-2250)	69[e]	(40-135)	3.5	2.0	9/10[e]	0/10	41.6[e]	11.6
Day 130 control					49	(41-60)	23	(12-47)	1.0	1.0	0/5	0/5	10.3	13.8
Day 214 DES	1378	1448	2.9	2.4	3147[e]	(1330-4900)	85[e]	(31-148)	4.5	2.0	9/10[e]	0/6	61.3[e]	12.8[f]
Day 214 control[g]					88	(14-413)	23	(10-50)	1.0	1.5	0/10	0/10	12.4	16.3

[a] Calculated instantaneous rate: μg/day = $dY/dX = 54.74\ X^{-0.5447}$; $Y = 120.23\ X^{0.4553}$ for ACI. μg/day = $dY/dX = 57.40\ X^{-0.5936}$; $Y = 141.25\ X^{0.4064}$ for S-D.

[b] On a scale of 1 to 5.

[c] Adenocarcinomas.

[d] Numbers in parentheses, range.

[e] Different from controls $p < 0.01$ ("t"-test).

[f] Different from controls $p < 0.05$ ("t"-test).

[g] These controls were implanted with a 20-mg pellet of cholesterol.

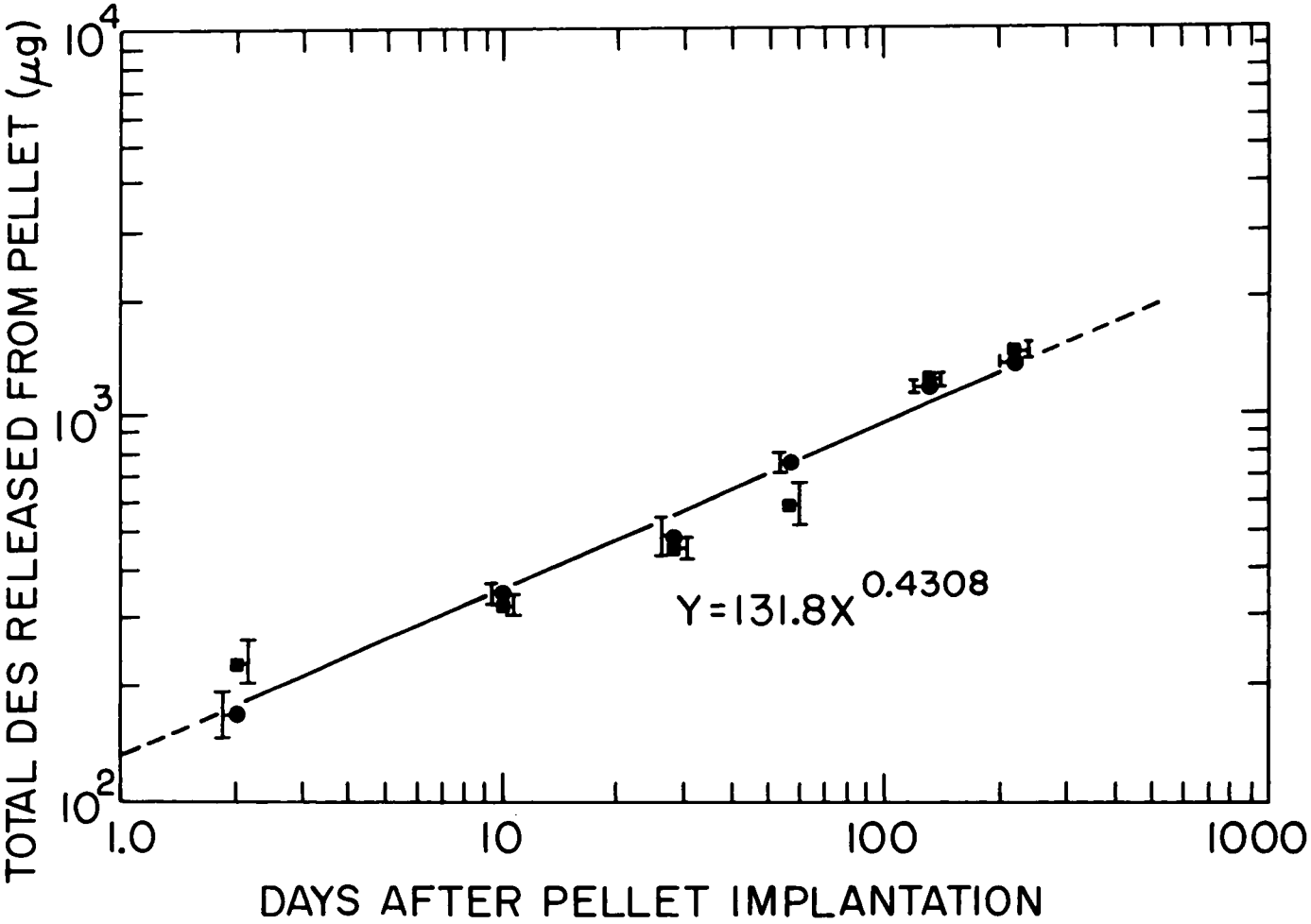

Chart 1. Release of DES from compressed pellet implanted in female ACI (●) or S-D (■) rats plotted as a function of time. Regression line was computed from the combined data (n = 111). *Bars*, S.E.

of Arthritis, Metabolism, and Digestive Diseases double antibody procedure. Briefly, the modifications were as follows: (*a*) the phosphosaline buffer (0.01 M, pH 7.6, plus 0.15 M NaCl and 0.1% NaN_3) used contained 1% egg albumin (Grade V; Sigma Chemical Co., St. Louis, Mo.) instead of 1% bovine serum albumin; (*b*) the ^{125}I-labeled prolactin, approximately 28,000 cpm/tube, was added on Day 1 rather than on Day 0. (*c*) the antiserum was used at a dilution of 1:34,000 instead of 1:25,000; (*d*) the iodination of the prolactin was done by using a lactoperoxidase method modified from the method of Thorell and Johansson (33), as described below. The reaction was carried out at room temperature (~22°) in a 1-ml conical reaction vial. The reactants were added in the following order and amounts: (*a*) 5 μg rat prolactin in 10 μl of 0.01 M $NaHCO_3$; (*b*) 10 μl of 0.5 M phosphate buffer, pH 7.6; (*c*) 1.0 to 1.5 mCi ^{125}I (10 to 20 μl; Amersham/Searle); (*d*) 10 μg (in 10 μl H_2O) lactoperoxidase (Sigma Chemical Co.); (*e*) 30 ng (10 μl) 0.88 mM H_2O_2. The mixture was stirred with a micromagnetic stirring bar for 10 min, after which the reaction was stopped by dilution with 500 μl of H_2O. To isolate the ^{125}I-prolactin, the reaction mixture was transferred to a 1- x 25-cm Sephadex G-75 (Pharmacia Fine Chemicals Inc., Piscataway, N. J.) column which had been pretreated with 5 ml of 1% human albumin fraction V (Sigma) and equilibrated with 0.01 M borate buffer, pH 8.6. The ^{125}I-prolactin was eluted from the column with borate buffer, and 1-ml fractions were collected in cold (4°) tubes containing 0.5 ml 1% egg albumin in phosphosaline buffer. The ^{125}I-prolactin in the first tube after the prolactin peak was used for the assays. This fraction was stored at 4° for up to 21 days and was repurified just prior to use by passing through a Sephadex G-75 column as above, except the phosphosaline buffer was used for elution.

RESULTS

DES Released from Pellet. An equation for the line representing the total DES released from the implanted pellets (Y) *versus* days after implantation (X) was calculated for each rat strain using a linear regression analysis of the logarithmically transformed data. For the ACI rats, the equation was $Y = 120.23X^{0.4553}$, and for the S-D rats it was $Y = 141.25X^{0.4064}$. Analyses of the data (t test) indicated that there were no significant differences between the amount of DES released from pellets implanted in either ACI or S-D rats; thus, the release of DES from the pellets in both strains could be represented by a single regression line, $Y = 131.8X^{0.4308}$ (correlation coefficient = 0.981, S.E. of estimate = 0.065). See Chart 1.

The instantaneous rate of DES release (μg/day) was computed for each sampling day for each strain using $dY/$

Table 2
Effect of DES pellet implantation on body weight, uterine weight, and ovarian weight

	Mean body wt (g)		Mean uterine wt (mg)		Mean ovarian wt (mg)	
Group	ACI	S-D	ACI	S-D	ACI	S-D
Day 0 control	147	210	253	392	60.4	81.1
Day 2 DES	138	213	289	521	47.8	86.4
Day 10 DES	136[a]	209	350	818[a]	51.1	74.8
Day 10 control	147	275	316	364	70.5	89.9
Day 28 DES	147	216	389	1063[a]	37.3[b]	55.6[a]
Day 28 control	157	242	339	528	57.3	97.7
Day 56 DES	154	219[a]	439[b]	1088[a]	39.2[b]	45.6[a]
Day 56 control	159	252	353	480	55.0	80.8
Day 130 DES	160[a]	214[a]	584	1130[a]	50.8	57.6[b]
Day 130 control	179	280	547	480	57.7	92.5
Day 214 DES	165[a]	218[a]	589	806	46.2	45.4
Day 214 control	186	279	538	664[c]	53.0	70.1

[a] Significantly different from controls; $p < 0.01$.
[b] Significantly different from controls; $p < 0.05$.
[c] Nine of 10 animals were in estrus.

dX as the rate function; for ACI rats, $dY/dX = 54.74X^{-0.5447}$; for S-D rats, $dY/dX = 57.40X^{-0.5936}$ (Table 1). The rate of release decreased exponentially, dropping by a factor of 7 between Day 2 and Day 56 but by a factor of only 2 between Day 56 and Day 214. Although the amount of DES released in either ACI or S-D rats was not significantly different, the actual dose per kg of body weight was significantly higher in the ACI rats from Days 10 through 214.

All pellets were surrounded by a tough connective tissue capsule by 10 days after implantation. By visual inspection, this capsule appeared to increase in thickness as the period of implantation increased.

Plasma Prolactin Values. Plasma prolactin levels in control female ACI rats ranged from a low of 5 ng/ml during diestrus to a high of 413 ng/ml at estrus (Table 1). In control female S-D rats, the ranges of plasma prolactin levels were from 5 ng/ml at diestrus to 50 ng/ml at estrus. In response to DES treatment, the mean prolactin level in ACI rats increased to almost 10 times control values after only 2 days and remained at this level through Day 28 (Table 1). By Day 56, the mean prolactin level had further increased to about 25 times control values. An additional increase was not seen until Day 214 when the mean prolactin level had risen to 35 times the control values. In contrast, mean prolactin levels in S-D rats increased to about only 3 times control values by Day 2; they remained at this level at Day 10 and then peaked at about 10 times the control values on Day 28. By Day 56, the mean prolactin level had decreased to about 7 times the control values. The prolactin level remained at this value through Day 130 and Day 214.

Body, Uterine, and Ovarian Changes. The mean body weight of the DES-treated ACI rats was significantly below that of the controls on Day 10 (Table 2). Thereafter, no significant differences in the mean body weight due to DES were seen until Day 130 and Day 214. In contrast, the mean body weight of DES-treated S-D rats was significantly below that of controls from Day 56 through Day 214.

The mean uterine weight of DES-treated ACI rats did not differ from controls except on Day 56 (Table 2). In contrast, significant increases in the uterine weights were seen in treated S-D rats from Day 28 through Day 130. Part of this increase was due to large amounts, up to 20 ml, of clear fluid in the uteri. On Day 56, 5 of 8 DES-treated S-D rats had uteri containing pus. By Day 214, 4 of 10 in the DES-treated S-D group had died because of pyometritis, and the remainder had pus within the uteri. No ACI rats had any evidence of a uterine infection.

Ovarian weights were depressed in DES-treated rats of both strains (Table 2). Although the duration of the depression appeared to be longer in the S-D rats, the degree of variability seen in the data precluded additional conclusions.

Pituitary Changes. In DES-treated ACI rats the mean pituitary weight had increased significantly by Day 28. This increase continued throughout the experiment, and by Day 214 the mean pituitary weight of treated ACI rats was 5 times that of controls. When examined histologically and immunohistochemically, pituitary tumors, from Day 56 onward, were found to be adenomas primarily composed of prolactin-type cells.

In DES-treated S-D rats, there were no significant increases in mean pituitary weight. In fact, on Day 214 there was a decrease in the mean weight of pituitaries from treated rats. For a complete description and discussion of pituitary changes, see the report of Holtzman *et al.* (14).

Mammary Development and Neoplasms. Most of the control ACI and S-D rats had mammary tissue which was scored as 1 or 2 on a developmental scale of 1 to 5 (Table 1). In ACI rats, DES stimulated both duct development and lobuloalveolar growth by Day 10, and this stimulation continued throughout the experiment. In the S-D rats, DES stimulated similar growth and development by Day 10, but the mammary tissue was less stimulated as the experiment continued. All mammary neoplasms seen in this study were classified as mammary adenocarcinomas and were found only in DES-treated ACI rats.

Mammary neoplasms were first noted in DES-treated ACI rats on Day 130 (Table 1). At this time, a total of 19 histologically confirmed mammary adenocarcinomas were found in 9 of the 10 treated rats. By Day 214, this total was 101 mammary adenocarcinomas in 9 of the 10 treated rats. No evidence of metastasis was seen.

DISCUSSION

Studies on the effects of continuous hormone treatment require an accurate determination of the rate of hormone release from the implant if valid statements about dose and effect are to be made. Although implanted hormone-cholesterol pellets have been widely used for continuous hormone treatment, few attempts have been made to measure hormone release accurately. Shimkin and White (29) and Lipschutz and Iglesias (20) estimated hormone release from the weight loss of pellets during implantation, but Dunning *et al.* (10) found that many of their pellets weighed the same or more at the end of the experiment. UV spectrophotometry was used to determine accurately the total amount of hormone remaining in recovered pellets (11), but this measurement gave no indication of the rate of hormone release during implantation. Kincl *et al.* (18) estimated the rate of release from [9,11-^{3}H]-19-norprogesterone-cholesterol pellets by monitoring the ^{3}H excreted in the urine and feces. This technique, however, provided only an approximation of the absorption rate because the parent steroid had been partially metabolized, and the metabolites may have been stored or excreted undetected through sweat and expired air (17). Joseph *et al.* (15) studied both the plasma levels and excretion of released hormone from progesterone-cholesterol pellets in rabbits and concluded that there was a zero-order release for 80 days. However, this technique probably did not measure accurately the rate of hormone release from the pellet because much of the original compound had been metabolized, and both metabolism and excretion rates may have changed during the experiment.

In the present study, the rate of DES release from DES-cholesterol pellets was ascertained by analyzing pellets removed at various time intervals after implantation. The DES released *versus* time data did not indicate a first-order reaction, linear on a semilog plot, as would be expected if the diffusion rate was dependent mainly on the pellet surface area. However, these data were linear on a log-log plot. The significance of this finding is not fully understood but probably indicates that DES release from the pellet was dependent primarily upon the increasing thickness and decreasing permeability of the fibrous connective-tissue capsule. This encapsulation of estrogen-containing pellets has been noted by several other investigators (1, 12, 17, 18, 29).

In the current experiment, the release of DES from pellets appeared to be dependent only upon the duration of implantation and was independent of the rat strain or body weight. Although the total DES released from the pellets was not different in either ACI or S-D rats, the DES dose per unit body weight was significantly higher in the ACI rats from Day 10 through 214, because ACI female rats are much smaller and lighter than S-D females of the same age. In spite of the known body weight differences, we chose to use pellets of the same weight in both strains to see if the rate of DES release was independent of strain and body weight. The strain specific responses to DES in this study are probably due to intrinsic organ sensitivity differences and are not dose related, because in another study using ACI rats we found that pellets containing as little as 1.67 mg of DES caused similar neoplastic responses (30).

In the current experiment, 3 very prominent strain differences in response to DES were noted: (*a*) DES produced significant uterine growth and pyometritis in S-D rats but not in ACI rats; (*b*) significant pituitary growth and subsequent prolactin-cell adenomas were seen in ACI but not in S-D rats; (*c*) mammary adenocarcinomas were found in ACI rats but not in S-D rats.

The finding of significant uterine growth only in treated S-D rats indicates that the uterine tissue of this strain is sensitive to exogenous estrogen, whereas ACI uterine tissue is relatively insensitive. The life-shortening pyometritis seen only in the DES-treated S-D rats appeared to be caused by a thickening of the cervical tissue, which prevented proper drainage of fluids from the uterus. Since infections and physiological stresses may suppress tumorigenesis, the pyrometritis might have been a factor contributing to the lack of mammary tumorigenesis in the DES-treated S-D rats.

In ACI rats, the DES treatment produced extremely high plasma prolactin levels in contrast to the modest increases seen in S-D rats. In female ACI rats, it is known that as the amount of DES in the pellet is increased the level of plasma prolactin increases (30). In female S-D rats, nothing has been reported regarding the plasma prolactin response to increasing amounts of DES in implanted DES-cholesterol pellets. Thus, it is possible that the DES-cholesterol pellet used in the present study was not optimal for elevating plasma prolactin levels in the female S-D rat during the experimental period. High prolactin levels in the treated ACI rats were accompanied by hypertrophy and hyperplasia of the prolactin cells of the adenohypophysis leading eventually to adenoma formation, but in the treated S-D rats stimulation of these cells was limited. These data demonstrate a definite strain difference in the stimulatory effects of DES on prolactin production and secretion.

The continued growth and maturation of breast tissue in DES-treated ACI rats could be correlated with the sustained high levels of prolactin but not with the decreased rate of DES release from the pellet. In the DES-treated S-D rats, the breast tissue stimulation noted between Days 10 and 28 also could be correlated with the elevated prolactin levels noted on these days. In addition, changes in circulating prolactin levels could be correlated with the efficient induction of mammary adenocarcinomas by DES in ACI rats and the lack of mammary neoplasia in S-D rats. In a study using similar DES pellets, Dunning *et al.* (9) found that 85% of the treated female ACI rats had mammary adenocarcinomas and significant pituitary weight increases but that less than 5% of the treated female Fischer or Copenhagen rats had any mammary tumors and that their pituitary weight increases were modest. The exceptional sensitivity of the female ACI rat to exogenous estrogen treatment may involve interactions of estrogen and prolactin. Estrogen can stimulate prolactin secretion by inhibiting the hypothalamic release of prolactin-inhibiting factor and by a direct stimu-

latory effect on pituitary prolactin cells (24). However, in female S-D rats it has been shown that very large doses of estrogen increased circulating prolactin levels but the growth of chemically induced mammary tumors was inhibited (22). The mechanism for this growth inhibition is thought to involve estrogen interference with prolactin binding to the mammary tissue (19, 23). This interference may be very low in ACI rats, or the extremely high prolactin levels induced by DES may overcome the estrogen interference.

It appears that control of pituitary prolactin secretion was the hormonal mechanism primarily responsible for the described strain-dependent mammary adenocarcinoma responses to DES. This conclusion is in agreement with the findings of other investigators: prolonged estrogen treatment is known to cause both pituitary and mammary tumors in several strains of rats (7, 10, 21, 31); and estrogen-induced pituitary adenomas have been shown to secrete large quantities of prolactin (13). The very important roles of prolactin (16, 22) and prolactin:estrogen ratios (2, 3, 4, 24) in mammary neoplasia have been stressed by several investigators. It seems that continuous stimulation of the breast tissue by sustained high levels of prolactin may be a necessary condition for mammary adenocarcinoma induction by estrogen treatment. Other hormonal factors may also be involved because many other hormones are known to affect mammary carcinogenesis (6). In addition, there may be a direct effect of DES on mammary adenocarcinoma induction, but this cannot be ascertained from the present data. In conclusion, it appears that the exceptional mammary neoplastic response of ACI rats to DES treatment is due primarily to the marked sensitivity of this strain to the induction of prolactin-secreting adenomas by DES.

It has been demonstrated previously that DES interacts synergistically with radiation on mammary adenocarcinoma formation in ACI rats (25) but not in S-D rats (28). The present finding that DES stimulates prolactin secretion to much higher levels in ACI rats than in S-D rats strongly suggests that the DES and radiation synergism seen in ACI rats must, in part, involve a synergistic interaction between elevated prolactin levels and radiation.

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