The Effects of Neonatal Androgenization on Mammary Gland Mitotic Rate and Susceptibility to Carcinogen-Induced Mammary Dysplastigenesis and Tumorigenesis in LEW/Mai Rats

Dallas M. Purnell, PhD

The influence of neonatal testosterone propionate treatment (androgenization) on mammary gland mitotic rate (MR) and susceptibility to 7,12-dimethylbenz(α)anthracene (DMBA) carcinogenesis was studied in female LEW/Mai rats. Mammary gland MR in androgenized rats was significantly lower than MR in normal rats at all ages studied. Treatment of androgenized rats with DMBA resulted in a significant increase in mammary gland MR in comparison with untreated androgenized rats. MR in DMBA-treated androgenized rats was similar to MR in DMBA-treated normal rats at most intervals after the introduction of the carcinogen. Although mammary epithelial MR in androgenized rats was significantly lower than that of normal rats of comparable age, no evidence of a decrease in susceptibility of mammary epithelium in androgenized rats to DMBA carcinogenesis was found. Instead, androgenized rats had a higher incidence of DMBA-induced mammary dysplasias, with no change in their morphologic or histologic features, than did normal rats; and there was no change in the incidence, latency, or histopathologic appearance of DMBA-induced mammary tumors in androgenized versus normal rats. (Am J Pathol 1980, 99:463–474)

A PERMANENT FUNCTIONAL ALTERATION in the integrity of the rodent hypothalmo-hypophyseal-ovarian axis can be effected by neonatal treatment with a single subcutaneous injection of testosterone propionate or estradiol benzoate.[1-4] This functional impairment results in an adult anovulatory syndrome characterized by absence of estrus cycle activity, persistent vaginal cornification, small polyfollicular ovaries lacking corpora lutea, infertility, and altered plasma levels of prolactin and estrogen.

Rodents exposed to sex steroids in the neonatal period also develop dysplastic and neoplastic lesions effecting vaginal and endometrial epithelia, as well as mammary gland abnormalities including duct ectasia, alveolar hyperplasias, and neoplasms.[5-8]

Neonatal mice exposed to estradiol or testosterone have an increased risk of developing mammary dysplasias and tumors when exposed to 7,12-dimethylbenz(a)anthracene (DMBA) as adults.[9] In contrast, neonatal androgenization of rats has generally been associated with a reduction in

the incidence of DMBA-induced mammary adenocarcinomas.[10-12] The effect of neonatal sex steroids on spontaneous or carcinogen-induced mammary dysplasia in adult rats has not previously been studied.

In the present study, we examined the effect of neonatal testosterone propionate administration on the replicative activity (mitotic rate) of mammary epithelium and on the susceptibility of mammary epithelium to DMBA-induced dysplastigenesis and tumorigenesis in the LEW/Mai rat. Our results indicate that neonatal testosterone treatment results in a significant depression of the mitotic rate of mammary epithelium that is not associated with a decrease in susceptibility to DMBA-induced carcinogenesis.

Materials and Methods

Animals

Normal and androgenized female LEW/Mai rats (N-rats and TP-rats) were obtained from Microbiological Associates, Bethesda, Maryland. Rats were androgenized by a single subcutaneous injection of 1.25 mg testosterone propionate (TP) (Sigma Chemical Company, St. Louis, Mo) dissolved in 0.05 ml sterile sesame oil at 5 days of age. The rats were shipped to our laboratory when they were 21 days of age. On receipt, they were maintained in air-conditioned rooms with 5 rats per cage under a light:dark cycle of 12 hours:12 hours. Food and water were supplied *ad libitum*.

Estrus Cycle Status

N-rats and TP-rats were examined daily to ascertain the time of vaginal opening. The time (days after birth) at which vaginal opening occurred in each rat was recorded and used to calculate a mean time of vaginal opening (±SE) in days for N-rats and TP-rats. After vaginal opening, smears of vaginal secretions were obtained daily, stained by the method of Papanicolaou, and used to ascertain estrus cycle activity by conventional cytologic criteria.

Carcinogen Treatment

At 52 days of age, groups of N-rats and TP-rats were anesthetized with ether, and 18 mg DMBA, dissolved in 1.0 ml of sesame oil, was administered by gastric gavage. DMBA-treated rats were housed 1 rat/cage over the first 5 days following DMBA feeding, and thereafter in groups of 5/cage.

Tissue Collection and Preparation

In the experiments groups of N-rats and TP-rats were killed between 82 and 172 days of age, and groups of DMBA-treated N-rats and TP-rats were killed at intervals between 7 and 80 days after exposure to the carcinogen. Six hours before the killing of the rats for tissue collection, each rat was given an intraperitoneal injection of colchicine (Calbiochem, Los Angeles, Calif) (1 mg/kg) in sterile saline. Whole body weight was measured at the time of death, and vaginal smears were obtained to ascertain the status of the estrus cycle. Both inguinal mammary gland fat pads with skin attached were removed from each rat and placed in Bouin's fixative. After overnight fixation, the inguinal mammary glands were removed from the skin. The right mammary glands were prepared as hematoxylin-stained whole mounts by the technique described elsewhere [13] and used to quantitate the

incidence of mammary dysplasias. The left mammary glands were dehydrated, embedded in paraffin, and used to prepare hematoxylin-stained histologic sections to assess mammary epithelial mitotic activity. In addition to the inguinal mammary glands, the pituitary gland and both ovaries were removed from each rat, weighed, and prepared for light-microscopic examination.

Mammary Dysplasias

The incidence of dysplasias in the mammary glands of rats was determined according to the total number of lesions revealed at low magnification per inguinal mammary gland whole mount. Data from individual rats killed at comparable ages or times after DMBA were aggregated and expressed as the mean number of dysplasias per positive inguinal mammary gland (±SE). The percentage of rats with one or more dysplasias per inguinal mammary gland at various ages and times after DMBA was also determined.

Two major dysplasias were observed: hyperplastic end buds (HEB) and hyperplastic alveolar nodules (HANs). The subgross and histologic features of these lesions in N-rats have been previously reported.[14] No obvious differences in the subgross or histologic appearance of dysplasias developing in N-rats and TP-rats were apparent.

Mitotic Activity

From each histologic section of mammary gland ten areas of lobuloalveolar epithelium were selected, by means of a previously described technique,[13] for the determination of mammary epithelial mitotic activity. For each mammary gland, mitotic activity was expressed as a 5-hour mitotic rate (number of mitoses/number of interphase nuclei + number of mitoses/5-hour colchicine blockade). Mitotic rates were multiplied by 100 to convert them to percentages. Mean mitotic rate for each experimental group (untreated N-rats and TP-rats and DMBA-treated N-rats and TP-rats) were obtained by aggregation of data obtained from rats of comparable age and, where possible (untreated and DMBA-treated N-rats), comparable estrus cycle status.

Tumor Incidence

Groups of N-rats and TP-rats were given a single dose (18 mg) of DMBA in sesame oil when they were 52 days of age. These rats were then allowed to live until they developed tumors. Rats were examined weekly, and the times of appearance and incidence of tumors were recorded. Histologic sections were prepared from tumors for microscopic examination.

Results

Vaginal Opening, Organ Weights, and Estrous Cycle Activity in N-Rats and TP-Rats

In TP-rats, vaginal opening occurred significantly earlier than in N-rats (32 ± 0.4 versus 39 ± 2 days, respectively, $P < 0.001$). The mean wet weight of ovaries obtained from N-rats was significantly higher than the mean weight of ovaries obtained from TP-rats at all time points (Table 1). In contrast, no significant differences were observed between N-rats and TP-rats in the case of mean pituitary weight or total body weight. Gross polycystic change was evident in some or all of the ovaries obtained from DMBA-treated TP-rats killed 50–70 days after the carcinogen. Such changes were not observed in ovaries obtained from DMBA-treated N-rats.

Table 1—Pituitary, Ovary, and Body Weights of N-Rats and TP-Rats at Different Ages and Intervals After DMBA Administration

Age (days)	Interval* after DMBA (days)	Pituitary Relative weight (mg/100 g)		Ovary Relative weight (mg/100 g)		Body weight (g)		Number of rats	
		N-rats	TP-rats	N-rats	TP-rats	N-rats	TP-rats	N-rats	TP-rats
82	—	10 ± 1†	8 ± 0.2	45 ± 2‡	17 ± 1	115 ± 2	194 ± 8	6	5
92	—	8 ± 1†	8 ± 0.1	32 ± 1†	21 ± 1	195 ± 6	207 ± 8	5	10
112	—	8 ± 0.4†	9 ± 0.1	25 ± 1‡	18 ± 1	238 ± 6	228 ± 5	6	10
132	—	7 ± 0.3†	8 ± 0.1	22 ± 1‡	23 ± 2	242 ± 21	239 ± 8	4	10
172	—	8 ± 1†	8 ± 0.1	24 ± 1‡	19 ± 1	235 ± 5	239 ± 10	4	5
66	14	8 ± 0.2†	9 ± 1	42 ± 0.2§	23 ± 1	182 ± 1	167 ± 9	34	5
82	30	8 ± 0.3†	8 ± 1	33 ± 0.1§	19 ± 1	202 ± 1	205 ± 15	31	5
92	40	8 ± 0.1†	8 ± 1	32 ± 0.3‡	23 ± 3	300 ± 1	204 ± 7	21	5
102	50	8 ± 0†	7 ± 1	28 ± 0.1§	18 ± 4	213 ± 1	240 ± 5	21	5
112	60	8 ± 0.1†	8 ± 0.4	29 ± 0.3‡	19 ± 2	212 ± 1	216 ± 16	19	5
122	70	8 ± 0†	7 ± 1	28 ± 0.1‡	16 ± 1	216 ± 1	239 ± 19	35	5

* 18 mg intragastrically at 52 days.
† $P > 0.05$, compared with the corresponding value for TP-rats.
‡ $P < 0.005$, compared with the corresponding value for TP-rats.
§ $P < 0.001$.

Vaginal smears from untreated TP-rats revealed large numbers of cornified squamous cells with a minor admixture of nucleated epithelial cells and polymorphonuclear leukocytes. Vaginal smears from DMBA-treated TP-rats were similar, but polymorphonuclear leukocytes were more numerous in such smears. No daily fluctuation in the vaginal cytology of untreated or DMBA-treated TP-rats was observed, which was consistent with the absence of normal estrus cycle activity in these rats. In contrast, untreated or DMBA-treated N-rats showed daily variation in vaginal smear cytology compatible with the regular 4-day estrus cycle activity in these animals.

Mitotic Rate of Mammary Epithelium in Untreated and DMBA-Treated N-Rats and TP-Rats

The mitotic rate of mammary epithelium in untreated TP-rats was significantly lower in comparison with untreated N-rats at most time points (Table 2). In addition, the mammary epithelial mitotic rate of untreated TP-rats remained constant relative to age, while untreated N-rats showed a significant increase in the mammary gland mitotic rate after about 90 days of age.

The mammary epithelial mitotic rate of DMBA-treated N-rats was similar to that of untreated N-rats, with the exception that the mitotic rate was significantly elevated at 30–40 days after DMBA. In contrast, the mitotic rate of mammary epithelium in DMBA-treated TP-rats was significantly higher than that of untreated TP-rats at all time points where comparisons were possible. The mitotic rate of mammary epithelium in DMBA-treated TP-rats and DMBA-treated N-rats was similar except at 60 and 70 days after the carcinogen; here it was significantly lower in TP-rats than in N-rats.

The mitotic rate of mammary epithelium in N-rats showed significant variation associated with the phase of the estrus cycle, as did that of DMBA-treated N-rats (Table 3). At intervals of less than 60 days after DMBA the mitotic rate at comparable cycle phases was generally higher in DMBA-treated N-rats than in untreated N-rats.

Mammary Gland Dysplasia in Untreated and DMBA-treated N-Rats and TP-Rats

Table 4 shows that 20–55% of untreated TP-rats at each time point examined had HANs, in contrast to untreated N-rats, where HANs were found only in the oldest rats examined. However, HEBs were not observed in untreated rats of either type.

Exposure to DMBA greatly increased the percentage of N-rats and TP-rats with HANs and, in addition, caused the appearance of a second mammary dysplasia (HEBs) in both types of rats. The percentage of rats with

Table 2—Mitotic Rate of Mammary Epithelium in Normal and Androgenized LEW/Mai Rats of Different Ages and at Different Times After DMBA Administration

Age (days)	Interval after DMBA* (days)	Untreated		DMBA-treated		Untreated vs DMBA-treated	
		N-rats	TP-rats	N-rats	TP-rats	N-rats	TP-rats
59	7	1.6 ± 0.2	—	1.5 ± 0.2	—	NS	
66	14	1.8 ± 0.7	—	2.6 ± 0.4	2.0 ± 0.5	NS	
82	30	1.9 ± 0.7 ‡	0.7 ± 0.2 ‡	2.8 ± 0.4	2.2 ± 0.2	P < 0.05	P < 0.001
92	40	1.9 ± 0.2 †	0.6 ± 0.1 ‡	3.1 ± 0.7	1.9 ± 0.4	P < 0.05	P < 0.001
102	50	3.7 ± 0.6	—	2.5 ± 0.4	2.4 ± 0.4 †	NS	
112	60	2.9 ± 0.3 ‡	0.6 ± 0.2 ‡	2.9 ± 0.4 ‡	1.4 ± 0.2	NS	P < 0.02
122	70	3.9 ± 0.5	—	3.9 ± 0.7 ‡	1.2 ± 0.4	NS	
132	80	3.6 ± 0.3 ‡	1.0 ± 0.3	3.2 ± 0.4	—		
172	—	2.7 ± 0.3	1.7 ± 0.4	—	—		

* 18 mg at 52 days.
† Within-column comparison, $P < 0.05$ (Student t test).
‡ Between-column comparison, $P \leq 0.05$, $P < 0.02$, $P < 0.001$, top to bottom, untreated; and $P < 0.05$, top to bottom, DMBA-treated (Student t test).
NS = not significant ($P > 0.05$) (Student t test).

Table 3—Mitotic Rate of Mammary Epithelium at Different Phases of the Estrus Cycle in Normal Untreated and DMBA-Treated LEW-Mai Rats

Cycle phase	Age/interval after DMBA* (days)							
	59/7		66/14		92/40		112/60	
	Untreated	DMBA	Untreated	DMBA	Untreated	DMBA	Untreated	DMBA
Proestrus	0.7 ± 0.4 ‡	1.0 ± 0.2	1.9 ± 1.6	2.0 ± 0.7	1.3 ± 0.6 ‡	2.4 ± 0.3	3.2 ± 0.6 ‡	2.5 ± 0.4
Estrus	0.9 ± 0.1 ‡	1.4 ± 0.3	1.4 ± 0.3 ‡	2.4 ± 1.0	1.3 ± 0.3 ‡	1.6 ± 0.2	3.0 ± 0.6 ‡	1.4 ± 0.2
Metestrus	2.0 ± 0.5	2.1 ± 1.0	2.4 ± 0.4	3.4 ± 0.7	1.9 ± 0.3 ‡	3.9 ± 0.9	3.5 ± 0.7	3.9 ± 0.7
Diestrus 1	2.9 ± 1.0 ‡	2.0 ± 0.6	—	2.1 ± 0.4	2.1 ± 0.6 ‡	3.2 ± 0.6	3.8 ± 1.2 ‡	2.6 ± 0.7
Diestrus 2	0.7 ± 0.5 ‡	1.8 ± 0.3	1.3 ± 0.2	—	2.5 ± 1.2	2.4 ± 0.6	2.5 ± 0.5	2.6 ± 0.7

* 18 mg intragastrically at 52 days.
† Within-column comparison, $P < 0.05$ (Student t test).
‡ Between-column comparison, $P < 0.05$ (Student t test).

Table 4—Percentage (Number) of Normal and Androgenized Rats with HEBs and HANs at Different Ages and at Different Times After DMBA Administration

	Age (days)	Interval after DMBA* (days)	% rats with			
			HEBs		HANs	
			N-rats	TP-rats	N-rats	TP-rats
Untreated						
	82	—	Not observed		0 (0/5)	40 (2/5)
	92	—	Not observed		0 (0/5)	20 (2/10)
	112	—	Not observed		0 (0/5)	55 (6/11)
	132	—	Not observed		0 (0/5)	40 (4/10)
	172	—	Not observed		20 (1/5)	20 (1/5)
DMBA-treated						
	66	14	80 (4/5)	60 (3/5)	0 (0/5)	0 (0/5)
	82	30	60 (3/5)	80 (4/5)	60 (3/5)	20 (1/5)
	92	40	40 (2/5)	0 (0/5)	80 (4/5)	80 (4/5)
	102	50	40 (2/5)	20 (1/5)	80 (4/5)	80 (4/5)
	112	60	0 (0/5)	20 (1/5)	100 (5/5)	100 (5/5)
	122	70	20 (1/5)	0 (0/5)	100 (5/5)	100 (5/5)

* 18 mg intragastrically at 52 days.

HEBs and HANs at various intervals after exposure to DMBA was similar in N-rats and TP-rats.

The incidence of HANs in untreated rats was low, particularly in N-rats (Table 5). Administration of DMBA greatly increased the incidence of HANs in both N-rats and TP-rats. At most intervals after DMBA, the incidence of HANs was significantly higher in TP-rats than in N-rats. HEBs appeared to be specifically related to carcinogen exposure, since they were not encountered in untreated rats of either type. Between 14 and 30 days after DMBA, the incidence of HEBs in TP-rats was significantly higher than in N-rats.

Mammary glands obtained from untreated and DMBA-treated TP-rats also had a high incidence of duct ectasia and showed signs of secretory activity, features which were not encountered in the mammary glands of N-rats. In addition, there appeared to be less lobulo-alveolar development in the mammary glands of TP-rats than in those of N-rats.

Mammary Tumors in DMBA-treated N-Rats and TP-Rats

As shown in Table 6, there were no significant differences in the final tumor incidence or time of appearance of tumors in N-rats and TP-rats exposed to DMBA at 52 days of age. Histologically all mammary tumors were adenocarcinomas of the papillary or cribriform type. However, milk secretion was more evident in mammary tumors from DMBA-treated TP-rats than those which developed in DMBA-treated N-rats.

Table 5—Incidence of Dysplasias in Inguinal Mammary Glands of Normal and Androgenized Rats of Different Ages and at Different Times After DMBA Administration

Age (days)	Interval after DMBA* (days)	Incidence† (range) per positive mammary gland of				Total number/positive mammary glands			
		HEBs		HANs		HEBs		HANs	
		N-rats	TP-rats	N-rats	TP-rats	N-rats	TP-rats	N-rats	TP-rats
Untreated									
82	—	Not observed	Not observed	0	1 ± 0	Not observed	Not observed	0/0	2/2
92	—	Not observed	Not observed	0	1 ± 0	Not observed	Not observed	0/0	2/2
112	—	Not observed	Not observed	0	1 ± 0.2 (1–2)	Not observed	Not observed	0/0	8/6
132	—	Not observed	Not observed	0	4 ± 2 (1–9)	Not observed	Not observed	0/0	16/4
172	—	Not observed	Not observed	2‡	2‡	Not observed	Not observed	2/1	2/1
Totals								2/1	30/15
DMBA-treated									
66	14	6 ± 2 (1–10) §	16 ± 1 (14–18)	0	0	24/4	49/3	0/0	0/0
82	30	5 ± 3 (1–9) ‖	19 ± 4 (13–25)	4 ± 3 (1–8)	1‡	14/3	71/4	12/3	1/1
92	40	5 ± 4 (2–8)	0	3 ± 1 (1–7)	9 ± 3 (5–15)	10/2	0/0	14/4	35/4
102	50	3 ± 2 (2–5)	5‡	2 ± 1 (1–5)	17 ± 4 (10–26) §	7/2	5/1	10/4	70/4
112	60	0	2‡	6 ± 2 (1–11)	13 ± 2 (8–18) ‖	0/0	2/1	31/5	67/5
122	70	1‡	0	7 ± 1 (5–10)	10 ± 1 (7–13) ¶	1/1	0/0	34/5	49/5
Totals						56/12	127/9	101/21	122/19

* 18 mg at 52 days.
† Mean ± SE.
‡ Single animal.
§ Comparison between columns, $P < 0.01$ (Student t test).
‖ Comparison between columns, $P < 0.05$ (Student t test).
¶ Comparison between columns, $P = 0.05$ (Student t test).

Table 6—Final Incidence of Mammary Tumors in Normal and Androgenized LEW/Mai Rats Exposed to DMBA

DMBA-treated*	% Rats with mammary tumors	Mean time of tumor appearance (days)
N-rats	40 (4/10)†	151 ± 14‡
TP-rats	30 (3/10)	177 ± 23

* 18 mg intragastrically at 52 days.
† $P > 0.05$, compared with TP-rats (Fisher's Exact Probability Test).
‡ $P > 0.05$, compared with TP-rats (Student t test).

Discussion

Our study has shown that exposure of neonatal female LEW/Mai rats to a single subcutaneous injection of testosterone propionate (1.25 mg) results in: 1) a significant reduction in mammary gland mitotic rate; 2) a significant increase in the incidence of spontaneous and DMBA-induced mammary dyplasias (but no change in their morphology or histologic features); and 3) no change in the incidence, latency, or histopathologic appearance of DMBA-induced mammary tumors.

To our knowledge, no previous studies have examined the effect of neonatal androgenization (TP) on mammary epithelial cell replication or dysplasia incidence in the rat. However, our results concerning the influence of neonatal TP on the incidence of mammary dysplasias in the rat coincide with those reported by Mori et al,[5] who found that neonatal Balb/cf C3H mice treated with TP had significantly more spontaneous mammary dysplasias in adult life than did normal mice. Similarly, neonatal Balb/c mice exposed to 17B estradiol and subsequently to DMBA as adults also had a higher incidence of mammary dysplasia than DMBA-treated normal mice.[9] On the other hand, the same investigators alluded to a stimulatory effect of neonatal 17B estradiol treatment on mammary epithelial cell proliferation that was in contrast to the effect of neonatal TP on rat mammary cell proliferation reported here. This finding may be indicative of a difference in response between the mammary gland of the mouse and that of the rat to neonatal hormone manipulation, since Nagasawa et al [15] reported that treatment of neonatal Sprague–Dawley rats with estrogen significantly retarded mammary gland growth.

In relation to the effect of neonatal TP-treatment of rats on mammary tumorigenesis, Shellabarger and Soo [10] and Christakos et al [11] reported a significant reduction in the incidence of mammary adenocarcinomas and an increased incidence of mammary fibroadenomas in neonatal androgenized Sprague–Dawley rats. Kovacs [12] also reported a reduction in the incidence of mammary tumors in neonatal androgenized Sprague–Dawley

rats, but the incidence of fibroadenomas relative to adenocarcinomas in these rats was not altered.

Our findings revealed no significant difference in mammary tumor incidence or latency between TP-rats and N-rats treated with DMBA. All tumors in both TP-rats and N-rats were adenocarcinomas.

The most interesting observation in the present study was the high incidence of mammary dysplasias and unaltered mammary tumor incidence observed in DMBA-treated TP-rats in spite of the fact that these rats had a significantly lower mammary gland mitotic rate at the time of carcinogen exposure. There is supporting evidence for a direct association between intensity of cell replicative activity at the time of carcinogen exposure and the incidence of dysplastic/neoplastic mammary lesions.[16-17] Nevertheless, our results suggest the existence of some other factor(s) apart from relative intensity of cell replicative activity at the time of carcinogen exposure that is instrumental in determining the final incidence of dysplasias and tumors.

References

1. Brown-Grant K, Munck A, Naftolin F, Sherwood MR: The effects of the administration of testosterone propionate alone or with phenobarbitone and of testosterone metabolites to neonatal female rats. Horm Behav 1971, 2:173–182
2. Barraclough C: Modifications in reproductive function after exposure to hormones during the prenatal and early postnatal period. Neuroendocrinology. Vol 2. Edited by L Martini, W Ganong. New York, Academic Press, 1967, pp 62–99
3. Mallampati R, Johnson DC: Serum and pituitary prolactin, LH and FSH in androgenized female and normal male rats treated with various doses of estradiol benzoate. Neuroendocrinol 1973, 11:46–56
4. Takewaki K: Some aspects of hormonal mechanism involved in persistent estrus in the rat. Experientia 1962, 18:1–6
5. Bern HA, Jones LA, Mori T, Young PN: Exposure of neonatal mice to steroids: Long-term effects on the mammary gland and other reproductive structures. J Steroid Biochem 1975, 6:673–676
6. Jones LA, Bern HA: Long-term effects of neonatal treatment with progesterone, alone and in combination with estrogen, on the mammary gland and reproductive tract of female BALB/cfC3H mice. Cancer Res 1977, 37:67–75
7. Mori T, Bern HA, Mills KT, Young PN: Long-term effect of neonatal steroid exposure on mammary gland development and tumorigenesis in mice. J Natl Cancer Inst 1976, 57:1057–1062
8. Nagasawa H, Mori T, Yanai R, Bern HA, Mills KT: Long-term effects of neonatal hormone treatments on plasma prolactin levels in female BALB/cfC3H and BALB/c mice. Cancer Res 1978, 38:942–945
9. Warner MR, Warner RL: Effects of exposure of neonatal mice to 17B estradiol on subsequent age-incidence and morphology of carcinogen-induced mammary dysplasia. J Natl Cancer Inst 1975, 55:289–298
10. Shellabarger CJ, Soo VA: Effects of neonatally administered sex steroids on 7,12-dimethylbenz(a)anthracene-induced mammary neoplasia in rats. Cancer Res 1973, 33:1567–1569

11. Christakos S, Sinha D, Dao TL: Neonatal modification of endocrine functions and mammary carcinogenesis in the rat. Br J Cancer 1976, 34:58–63

12. Kovács K: Effect of androgenisation on the development of mammary tumours in rats induced by the oral administration of 9,10-dimethyl-1,2-benzanthracene. Br J Cancer 1965, 19:531–537

13. Purnell DN, Stowers DJ: Mammary gland hyperplastic alveolar noduligenesis in the LEW–Mai rat in relation to mitotic activity, estrous cycle status, and age. J Natl Cancer Inst 1978, 61:1339–1346

14. Purnell DM: Relationship of terminal duct hyperplasia to mammary carcinoma in 7,12-dimethylbenz(α)anthracene-treated LEW/Mai rats. Am J Pathol 1980, 98:311–324

15. Nagasawa A, Yanai R, Shodono M, Nakamura T, Tanabe Y: Effect of neonatally administered estrogen or prolactin on normal and neoplastic mammary growth and serum estradiol 17B level in rats. Cancer Res 1974, 34:2643–3646

16. Nagasawa H, Yanai R, Taniguchi H: Importance of mammary gland DNA synthesis on carcinogen-induced mammary tumorigenesis in rats. Cancer Res 1976, 36:2223–2226

17. Nagasawa H, Yanai R: Frequency of mammary cell division in relation to age: Its significance in the induction of mammary tumors by carcinogen in rats. J Natl Cancer Inst 1974, 52:609–610