Effects of High Doses of 17 β -estradiol and Ovariectomy on 7, 12-Dimethylbenz[a]anthracene-Induced Mammary Carcinomas and Dysplasias in Neonatally Androgenized Female Sprague-Dawley Rats

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Abstract: Inbred, female Sprague-Dawley rats were neonatally androgenized at the age of 2 days by subcutaneous injections of 1.25 mg testosterone propionate and divided into 4 groups. At the age of 50 days, rats of all groups were given 20 mg 7, 12-dimethylbenz[a]anthracene (DMBA). Four weeks after the administration of DMBA, the rats in Group I remained intact, those in Group II were subjected to ovariectomy, while the animals in Groups III and IV were given 1 mg 17 β -estradiol (E₂) at 2-day intervals for 3 weeks. Additionally, the rats in Group IV were given 1 mg E₂ at 2-day intervals for 3 weeks by intramuscular injection, starting from the 105th day after the administration of DMBA.

The ovaries of the neonatally androgenized rats contained numerous large vesicular follicles and no corpora lutea, and the mammary glands of the animals in Groups I, III and IV consisted mainly of dilated acini containing milky secretions. In rats in Group I, mammary carcinomas and mammary dysplasias were induced. Rat mammary dysplasias were grossly characterized by gross cysts and solid masses. Solid masses were heteromorphic lesions and commonly consisted of several nodules with various microscopic features. These masses were subclassified into the 2 types of histological features that were predominant among the nodules: acinar adenosis and fibrotic adenosis.

The induction of mammary carcinomas and mammary dysplasias was strongly suppressed by ovariectomy (Group II) as the development of mammary carcinomas and mammary dysplasias induced in neonatally androgenized rats are dependent on estrogens. The induction of mammary carcinomas was suppressed by injections of high doses of E₂ for 21 days (Once in Group III and twice in Group IV). Conversely, the induction of mammary dysplasias was promoted by one time (Group III) but was suppressed by two times (Group IV) of E₂. The results indicate that carcinoma cells and dysplasia cells induced by DMBA in neonatally androgenized rats differ in response to high doses of E₂. (*J Toxicol Pathol* 2000; 13: 77~85)

Key words: mammary carcinoma, mammary dysplasia, 7, 12-dimethylbenz[a]anthracene, 17 β -estradiol, progesterone, rat

Introduction

Mammary dysplasia is termed a fibrocystic disease, and chronic cystic mastopathy and chronic cystic mastitis, are common diseases of the breast in women of middle age, characterized by gross cysts and solid masses. Solid masses consist of several nodules with various microscopic features such as adenosis, fibrosis, microcysts and ductal hyperplasia. In patients with mammary dysplasia particularly which includes ductal hyperplasia, the risk of mammary cancer was significantly greater than in women of the same age without mammary dysplasia¹⁻⁴. There have been many studies on mammary carcinogenesis, and some reports on reproductive risk factors relating to mammary carcinomas have indicated that long periods without breastfeeding is associated with the risk of mammary carcinomas⁵⁻¹³. Studies on the pathogenesis of mammary dysplasia, however, have been few and

the relationship between mammary dysplasia and lactation has not been clarified.

We have reported that a rat mammary disorder is induced in neonatally androgenized rats by the administration of 7,12-dimethylbenz[a]anthracene (DMBA)¹⁴⁻¹⁸. Two types of masses, solid and cystic, are grossly observable in those rats. Morphologic feature of that disorder, as revealed by careful histologic examination, sufficiently satisfies the criteria of human mammary dysplasia¹⁶. The primary lesion of rat mammary dysplasia is an abnormal alveolar nodule with histological features of acinar adenosis¹⁵. Acinar adenosis evolves mainly into fibrotic adenosis with increased stromal connective tissues. The histologic feature observed in those rats with cystic lesions, i.e., progressively unfolding lobules in which acini are distended or coalesced and contained milky secretions suggests that the contained accumulation of milky secretion may be the major factor in cyst formation. The objective of this investigation was to elucidate the effects of lactation induced by injections of high doses of 17 β -estradiol (E₂) on the progression of rat mammary dysplasia and mammary carcinoma.

Material and Methods

Sprague-Dawley rats that were bred in our laboratory,

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Fig. 1. Protocol and Group

were maintained in an air-conditioned room at $25 \pm 2^\circ\text{C}$ and given commercial diet (Oriental Yeast Co., Tokyo) and tap water *ad libitum*. This study was carried out in accordance with Guideline for Animal Experimentation, Faculty of Medicine, Kagoshima University. All the female rats were neonatally androgenized at the age of 2 days by subcutaneous injections of 1.25 mg testosterone propionate (TP) (Sigma Chemical Co., St. Louis, Mo.) in 0.05 ml sesame oil and hysterectomized at the age of 28 days. These rats (TP rats) were then divided into 4 groups. At the age of 50 days, the rats of all groups were given 20 mg 7, 12-dimethylbenz[a]anthracene (DMBA) (Wako Pure Chemical Co., Osaka) dissolved in 1 ml sesame oil, by gastric intubation. Four weeks after the administration of DMBA, the rats in group II were ovariectomized (OVEX), while rats in group III and IV were given 1 mg 17 β -estradiol (E_2) (Sigma Chemical Co.) at 2-day intervals for 3 weeks and rats in group IV were given 1 mg E_2 at 2-day intervals for 3 weeks by intramuscular injections starting from the 105th day after the administration of DMBA. The treatments given to the rats in the various groups are clearly shown in Fig. 1.

The animals were palpated for mammary masses once a week, and were decapitated on day 264, i.e., at the age of 314 days. Mammary masses were excised, sectioned, and examined histologically. Wet weights of the pituitary and adrenal glands, and ovaries were taken on a precision balance immediately following dissection.

Pituitaries were fixed in 10% phosphate-buffered formalin (PBF) at room temperature and embedded in paraffin. Sections were cut from paraffin embedded tissue blocks, deparaffinized and rehydrated. They were subjected to the standard avidin-biotin complex immunoperoxidase assays using the Vectastain ABC kit (Vector Laboratories, USA). Deparaffinized and rehydrated sections were treated with 0.3% hydrogen peroxide in methanol solution to block endogenous peroxidase activity. They were placed in phosphate-buffered saline (PBS), and were subsequently incubated at room temperature with the following reagents (with PBS washes in between): 1.0% normal bovine serum albumin (BSA)-PBS-NP40 for 30 min. for blocking, primary antibody, prediluted rabbit anti-human prolactin (DAKO CORPORATION, USA) for 60 min., biotinylated goat anti-rabbit immunoglobulin (IgG) for 60 min., ABC complex for 60 min., and 3,3'-diaminobenzidine tetrachloride (DAB) con-

taining 0.05% hydrogen peroxide for 5 min. The sections were then washed, counter-stained, dehydrated, cleared in xylene, and mounted.

Mean differences were evaluated by Student's *t*-test. The incidence of tumors and the total number of mammary tumors in each group were tested by means of the Fourfold Contingency Table¹⁹.

Results

Effects of Neonatal Androgenization and additional injections of E_2 on mammary glands, ovaries and pituitaries

The mammary glands in Groups I and III consisted mainly of numerous dilated acini containing milky fluid and lined with a single layer of epithelial cells.

None of the ovaries of androgen-treated rats contained corpora lutea, but numerous large vesicular follicles and interstitial cells were observed. (Table 1)

Immunohistochemical examination of pituitaries of androgenized rats revealed that prolactin-positive cells increased in Groups III and IV (Fig. 2), compared with that of Group I (Fig. 3).

Endocrine Organ Weights

14 of 44 rats in Group I, 1 of 15 rats in Group II, 16 of 47 rats in Group III and 8 of 15 rats in Group IV died by the end of experiment.

Weights of adrenals and pituitary glands in Group II were significantly low ($p < 0.01$ and 0.01 , respectively), when compared to those in Group I, and weights of the pituitary glands in Groups III and IV were significantly high ($p < 0.01$ and 0.01 , respectively), when compared to those in Group I. (Table 1)

Pathology of masses in Androgenized Rats with oral administration of DMBA

Two types of palpable tumor-forming lesions were found in the mammary glands of rats androgenized neonatally and treated by oral administration of DMBA: mammary carcinoma and mammary dysplasia. (Table 2)

The number of rats with mammary carcinoma in Groups II and IV, and the multiplicity of mammary carcinoma in Groups II, III and IV were significantly low ($p <$

Fig. 2. Pituitary of a neonatally androgenized rat with two times of injections of E_2 . Immunohistochemical method. $\times 690$

Fig. 4. Solid tumor of various features of acinar adenosis and fibrotic adenosis in a neonatally androgenized rat treated with DMBA. H & E. $\times 50$

Fig. 3. Pituitary of a neonatally androgenized rat without injections of E_2 . Immunohistochemical method. $\times 690$

Fig. 5. Fibrotic adenosis. H & E. $\times 70$

Table 1. Effect of various hormonal treatment on endocrine organs in neonatal androgenized female Sprague-Dawley rats

Group	Hormonal treatment [§]	Initial No. of rat	No. of rats	Organ weight (mg, mean $\pm$ SD)			No. (%) of rats with corpora lutea in ovaries
				Adrenal glands	Pituitary gland	Ovaries	
I	TP	44	30	48.4 $\pm$ 9.0	28.9 $\pm$ 9.0	37.6 $\pm$ 9.8	0 (0)
II	TP+OVEX	15	14	29.2 $\pm$ 4.5**	13.3 $\pm$ 1.4**	—	—
III	TP+1 mg E ₂ (3 weeks)	47	31	48.5 $\pm$ 9.4	35.2 $\pm$ 7.4**	37.1 $\pm$ 12.8	0 (0)
IV	TP+1 mg E ₂ (3 weeks+3 weeks)	15	7	40.6 $\pm$ 11.0	95.4 $\pm$ 69.0***#	35.5 $\pm$ 22.3	0 (0)

[§] At 50 days of age, 20 mg DMBA was given to rats of all groups by gastric intubation; 1.25 mg testosterone propionate (TP) was given by s.c. injection at 2 days of age; ovariectomy (OVEX) was done 4 weeks after the administration of DMBA; 1 mg 17 β -estradiol (E₂) was given 3 times in a week by i.m. injection starting from 4 weeks or 15 weeks after the administration of DMBA.

**differs from Group I; $p < 0.01$

differs from Group III; $p < 0.01$

Table 2. Effect of various hormonal treatment on DMBA-induced mammary carcinoma and mammary dysplasia in neonatally androgenized female Sprague-Dawley rats

Group, treatment [§] and diagnosis		Initial No. of rats	No. of rats	No. (%) of rats with masses	No. of masses	No. of masses per rat $\pm$ SD	Mean size (cm ²) of masses per rat $\pm$ SD	Mean day of detection $\pm$ SD
I	TP	44	30					
	Mammary carcinoma			28 (93.3)	109	3.6 $\pm$ 2.4	3.9 $\pm$ 4.2	133.3 $\pm$ 44.9
	Mammary dysplasia			16 (53.3)	56	1.9 $\pm$ 2.5	1.8 $\pm$ 2.1	178.9 $\pm$ 43.3
II	TP+OVEX	15	14					
	Mammary carcinoma			3 (21.4)**	3	0.2 $\pm$ 0.4**	4.5 $\pm$ 3.9	184.0 $\pm$ 62.6
	Mammary dysplasia			0 (0.0)**	0	0**	—	—
III	TP+1mg E ₂ (3weeks)	47	31					
	Mammary carcinoma			24 (77.4)	53	1.7 $\pm$ 1.8**	4.8 $\pm$ 4.4	195.4 $\pm$ 51.0**
	Mammary dysplasia			25 (80.6)*	127	4.1 $\pm$ 4.3**	2.0 $\pm$ 2.6	229.6 $\pm$ 34.3**
IV	TP+1mg E ₂ (3weeks+3 weeks)	15	7					
	Mammary carcinoma			4 (57.1)*	7	1.0 $\pm$ 1.0**	4.0 $\pm$ 5.4	191.0 $\pm$ 60.3**
	Mammary dysplasia			4 (57.1)	10	1.4 $\pm$ 1.6 #	1.3 $\pm$ 1.5	223.9 $\pm$ 39.2**

[§] At 50 days of age, 20 mg DMBA was given to rats of all groups by gastric intubation; 1.25 mg testosterone propionate (TP) was given by s.c. injection at 2 days of age; ovariectomy (OVEX) was done 4 weeks after the administration of DMBA; 1 mg 17 β -estradiol (E₂) was given 3 times in a week by i.m. injection starting from 4 weeks or 15 weeks after the administration of DMBA.

* differs from Group I; $p < 0.05$

**differs from Group I; $p < 0.01$

differs from Group III; $p < 0.01$

0.01 and 0.05, and 0.01, 0.01 and 0.01, respectively), when compared to those in Group I. The latent period of mammary carcinoma in Groups III and IV was protracted significantly ($p < 0.01$ and 0.01, respectively), when compared to that in Group I.

No mammary dysplasia was induced in the rats of Group II. The number of rats with mammary dysplasia and the multiplicity of mammary dysplasia increased significantly ($p < 0.05$ and 0.01, respectively) in Group III but not in Group IV, when compared to those in Group I. However, the latent period of mammary dysplasia in rats of Groups III and IV was protracted significantly ($p < 0.01$ and 0.01, respectively), when compared to that in Group I. The incidence of mammary dysplasia decreased significantly ($p < 0.01$) in Group IV, when compared to that in Group III.

Pathology of mammary dysplasia in Androgenized Rats by DMBA

Rat mammary dysplasias were grossly characterized by gross cysts and solid masses. A solid mass of mammary dysplasia (heteromorphic lesions) commonly consisted of

several nodules with various microscopic features. These masses were subclassified into the 2 types of histological features that were predominant in most of the nodules: acinar adenosis and fibrotic adenosis. (Table 3)

Most of the acinar adenosis masses were small, yellow, and soft; and accordingly, they were difficult to detect by palpation. The masses commonly consisted of a few nodules of acinar adenosis that were of a large lobular structure, composed of numerous acini. The lumina of the acini were dilated and filled with acidophilic secretions in the rats of Groups I, III and IV and the epithelial linings of those lumina in Groups I, III and IV were compelial atypism, or modification of the stroma either in or around the nodule were observed. (Fig. 4)

Fibrotic adenosis masses, by far the commonest type in rats with mammary dysplasia, were whitish yellow and firmer than acinar adenosis masses. Fibrotic adenosis consisted of ample hyalinized stroma and developed acini. (Fig. 5)

Gross cysts were soft, yellow, oval, and filled with milky fluid. Frequently, some cysts were masses 5 $\times$ 4 cm. Larger cysts were palpable and grossly visible. Microscopically,

Table 3. Effect of various hormonal treatment on DMBA-induced mammary dysplasia in neonatally androgenized female Sprague-Dawley rats

Group, treatment ^s and diagnosis	Initial No. of rats	No. of rats	No. (%) of rats with Masses	No. of masses	No. of masses per rat $\pm$ SD	Mean size (cm ²) of masses per rat $\pm$ SD	Mean day of detection $\pm$ SD
I TP	44	30					
Solid tumor			12 (40.0)	45	1.5 $\pm$ 2.4	2.0 $\pm$ 2.3	186.2 $\pm$ 38.1
Acinar adenosis			1 (3.3)	4	0.1 $\pm$ 0.7	0.9 $\pm$ 0.6	230.3 $\pm$ 27.5
Fibrotic adenosis			12 (40.0)	41	1.4 $\pm$ 2.1	2.1 $\pm$ 2.4	181.9 $\pm$ 36.5
Gross cyst			9 (30.0)	11	0.4 $\pm$ 0.7	0.8 $\pm$ 0.8	149.3 $\pm$ 52.1
II TP+OVEX	15	14					
Solid tumor			0 (0.0)**	0	0**	—	—
Acinar adenosis			0 (0.0)	0	0	—	—
Fibrotic adenosis			0 (0.0)**	0	0**	—	—
Gross cyst			0 (0.0)*	0	0**	—	—
III TP+1mg E ₂ (3 weeks)	47	31					
Solid tumor			22 (71.0)*	77	2.5 $\pm$ 2.9	2.1 $\pm$ 2.5	231.0 $\pm$ 33.4**
Acinar adenosis			10 (32.3)**	18	0.6 $\pm$ 1.0	1.9 $\pm$ 1.9	243.7 $\pm$ 17.3
Fibrotic adenosis			20 (64.5)	59	1.9 $\pm$ 2.3	2.1 $\pm$ 2.6	227.1 $\pm$ 36.2**
Gross cyst			21 (67.7)**	50	1.6 $\pm$ 1.9**	1.8 $\pm$ 2.7	227.4 $\pm$ 35.9**
IV TP+1 mg E ₂ (3 weeks+3 weeks)	15	7					
Solid tumor			2 (28.6) [#]	3	0.4 $\pm$ 0.8 ^{##}	2.3 $\pm$ 2.7	223.7 $\pm$ 21.0
Acinar adenosis			0 (0.0)	0	0 ^{##}	—	—
Fibrotic adenosis			2 (28.6)	3	0.4 $\pm$ 0.8 ^{##}	2.3 $\pm$ 2.7	223.7 $\pm$ 21.0
Gross cyst			3 (42.9)	7	1.0 $\pm$ 1.4	0.9 $\pm$ 0.6 [#]	224.0 $\pm$ 46.4**

^s At 50 days of age, 20 mg DMBA was given to rats of all groups by gastric intubation; 1.25 mg testosterone propionate (TP) was given by s.c. injection at 2 days of age; ovariectomy (OVEX) was done 4 weeks after the administration of DMBA; 1 mg 17 β -estradiol (E₂) was given 3 times in a week by i.m. injection starting from 4 weeks or 15 weeks after the administration of DMBA.

* differs from Group I; $p < 0.05$

**differs from Group I; $p < 0.01$

[#] differs from Group III; $p < 0.05$

^{##} differs from Group III; $p < 0.01$

Table 4. Effect of various hormonal treatment on DMBA-induced microscopic lesions in neonatally androgenized female Sprague-Dawley rats

Group and treatment ^s	Initial No. of rats	No. of rats	No. (%) of rats with			No. of Microcarcinoma per rat $\pm$ SD	No. of Hyperplasia per rat $\pm$ SD	No. of Microcyst per rat $\pm$ SD
			Microcarcinoma	Hyperplasia	Microcyst			
I TP	44	30	5 (16.7)	11 (36.7)	18 (60.0)	0.23 $\pm$ 0.57	0.50 $\pm$ 0.78	1.47 $\pm$ 1.68
II TP+OVEX	15	14	0 (0.0)	0 (0.0)**	0 (0.0)**	—	—	—
III TP+1 mg E ₂ (3 weeks)	47	31	9 (29.0)	20 (64.5)*	17 (54.8)	0.38 $\pm$ 0.67	1.74 $\pm$ 2.25**	1.55 $\pm$ 2.19
IV TP+1 mg E ₂ (3 weeks+3 weeks)	15	7	2 (28.6)	2 (28.6)	1 (14.3)	0.29 $\pm$ 0.49	0.43 $\pm$ 1.13 [#]	0.14 $\pm$ 0.38** [#]

^s At 50 days of age, 20 mg DMBA was given to rats of all groups by gastric intubation; 1.25 mg testosterone propionate (TP) was given by s.c. injection at 2 days of age; ovariectomy (OVEX) was done 4 weeks after the administration of DMBA; 1 mg 17 β -estradiol (E₂) was given 3 times in a week by i.m. injection starting from 4 weeks or 15 weeks after the administration of DMBA.

* differs from Group I; $p < 0.05$

**differs from Group I; $p < 0.01$

[#] differs from Group III; $p < 0.05$

almost all the cysts were epithelial, with walls lined by a single layer of flattened or cuboidal epithelium and with sclerotic stroma. Epithelial cell proliferation leading to the accumulation of masses or to small papillary excrescences was common in these cysts, and they often contained amorphous, granular stroma. (Fig. 6)

The number of rats with solid tumors, acinar adenosis masses, gross cysts, and the multiplicity of gross cysts in Group III increased significantly ($p < 0.05$, 0.01, 0.01, and $p < 0.01$, respectively), when compared to those in Group I. (Table 3)

The number of rats with solid tumors of mammary

Fig. 6. Gross cyst. H & E. $\times 30$

Fig. 8. Microcarcinoma. H & E. $\times 50$

Fig. 7. Hyperplasia. H & E. $\times 140$

Fig. 9. Microcyst. H & E. $\times 70$

dysplasia, the multiplicity of masses of solid tumors, acinar adenosis, fibrotic adenosis, and the sizes of gross cysts in Group IV decreased significantly ($p < 0.05$, $p < 0.01$, 0.01 , 0.01 , and 0.05 , respectively), when compared to those in Group I. The latent periods of solid tumors of mammary dysplasia, fibrotic adenosis masses in Group III and gross cysts in Groups III and IV were significantly ($p < 0.01$, 0.01 , $p < 0.01$ and 0.01 , respectively) long, when compared to those in Group I.

Microscopic lesions in Androgenized Rats

Besides these macroscopically detectable tumor-forming lesions, three microscopic lesions were observed in some masses of mammary dysplasia and mammary glands: hyperplasia, microcarcinoma, and microcysts. (Fig. 7-9) Those microscopic lesions were not induced in rats of Group II. In Group III, the number of rats with hyperplasia and the multiplicity of hyperplastic lesions increased significantly ($p < 0.05$ and 0.01 , respectively), when compared to those in Group I. In Group IV, the number of rats with microcysts and the multiplicity of microcysts decreased significantly ($p < 0.05$ and 0.01 , respectively), when compared to those in Group I. The number of microcysts and the multiplicity of hyperplastic lesions and microcysts in Group IV decreased significantly ($p < 0.05$ and 0.05 , respectively), when compared to those in Group III.

Discussion

In the all neonatally androgenized female rats, corpora lutea were absent from the ovaries, although numerous large vesicular follicles were present. These changes in neonatally androgenized rats have been considered to be due to a disturbance of the gonadotropin-secreting system in the hypothalamus^{21,22}. Inasmuch as estrous cycles are found in neonatally intact female rats but not in neonatally androgenized female rats, it is difficult to compare hormone levels of intact and androgenized rats. According to previous studies, the levels of serum prolactin and estrogen in neonatally androgenized rats seem to be within the normal range in neonatally intact rats, although the level of serum progesterone is much lower in neonatally androgenized rats than in neonatally intact rats^{17,20}.

Our previous reports have indicated that the induction of mammary carcinomas by the administration of DMBA is strongly suppressed in those neonatally androgenized rats, whereas the induction of mammary dysplasia is significantly accelerated in comparison with the neonatally intact rats¹⁴⁻¹⁸. The degree of induction of mammary carcinomas and dysplasias in neonatally androgenized rats between our present and previous studies differs. The difference appears to be due to differences in the strains of the rats used, and differences in the periods of observation.

The induction of mammary carcinomas and mammary dysplasia was strongly suppressed in the androgenized rats that were ovariectomized (Group II). The results confirm our previous study showing that the development of rat mammary carcinomas and mammary dysplasias induced by DMBA in neonatally androgenized rats is dependent on estrogens¹⁶⁻¹⁸. Estrogen-dependency of those rat disorders is in accord with that of human mammary carcinomas and dysplasias, because they are not diseases of the male breast nor of ovariectomized women²³. We have reported that the growth of DMBA-induced mammary carcinomas is suppres-

sed by ovariectomy, which induces an inhibitory effect on the proliferation of carcinoma cells²⁴.

The induction of mammary carcinomas in neonatally androgenized rats was suppressed by injections of high doses of E_2 for 21 days at both one time (Group III) and two times (Group IV). We have reported that the growth of DMBA-induced mammary carcinomas in neonatally intact female and male rats was inhibited by injections of high doses of E_2 ²⁴⁻²⁸. In rats treated with injections of high doses of E_2 , the incidences of apoptotic cells and Apop Tag positive cells became higher than those of intact control rats from 7 days after the initial injection. According to findings in previous studies²⁴⁻²⁸, there is a possibility that apoptosis in the cells of some early carcinomas induced by DMBA in neonatally androgenized rats may be accelerated by injections of high doses of E_2 soon after the initial injections. Conversely, the induction of mammary dysplasias in neonatally androgenized rats was promoted at one time of the 21 day-injections (Group III) but was suppressed at two times (Group IV). The results indicate that carcinoma cells and dysplasia cells induced by DMBA in neonatally androgenized rats differ in response to high doses of E_2 . In Group III, incidences of solid tumor of acinar adenosis, gross cysts and microscopic lesions of hyperplasia increased, compared with those in Group I and in Group IV, the multiplicity of solid tumors of acinar adenosis or fibrotic adenosis and microscopic lesions of hyperplasia and microcysts were reduced, compared with those in Group III. It has been shown that in normal mammary glands of neonatally intact rats the percentage of the breast volume occupied by gland parenchyma decreases rapidly after weaning, falling from 88% at the time of weaning to approximately 10% at 14 days, and that involution of the lactating mammary glands following weaning is due to cell death by apoptosis²⁹. Falling circulating levels of prolactin have been implicated as one of causes of mammary gland involution after weaning³⁰⁻³³. In the androgenized rats (Groups III and IV) of this study, according to those features of mammary glands with accumulations of milky secretions in dilated lumina and pituitaries with large numbers of prolactin-positive cells (Fig. 2) and results of previous studies on the relationship between estrogen and prolactin^{34,35}, serum levels of prolactin seem to be much higher during the period when injections of E_2 are given than in the period when no injections are given.

According to the results of this and previous studies^{14,17,18}, we speculate that the reason why one time of injections of high doses of E_2 promotes induction of mammary dysplasia may be that progression of almost all of the minimum changes induced by DMBA in mammary glands into a variety of early lesions such as acinar adenosis of mammary dysplasia may extremely accelerate during the initial injections of high doses of E_2 , although some of the early lesions of mammary dysplasias may regress by apoptosis of mammary dysplasia cells due to falling levels of prolactin following the cessation of injections. We also speculate that the reason why the latent periods of mammary dysplasias in Groups III and IV become longer may be that high doses of E_2 may progress minimum changes which cannot progress in neonatally androgenized rats without high doses of E_2 (in Group I). Suppression of induction of mammary dysplasia by two times of injections seems to be due to the second falling levels of prolactin-induced apoptosis of many mammary dysplasia cells which may survive in the first condition with falling levels of prolactin (Group IV), although further

studies on the apoptosis of rat mammary dysplasia in various hormonal conditions and examination of serum levels of prolactin are necessary. A possibility that the effect of high doses of E₂ on development of mammary dysplasia might be a toxic effect can not be completely ruled out, since a considerable number of rats died in neonatally androgenized rats with injections of high doses of E₂. Therefore, the toxicological study is also necessary.

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