Virchows Archiv A Pathol Anat (1988) 414:1–7

Virchows Archiv A
Pathological Anatomy and Histopathology

Morphological and biological characteristics of mammary tumours induced by the direct application of DMBA powder to rat mammary glands*

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Summary. Mammary tumours were induced by the direct dusting of 1 mg, 7,12-dimethylbenz(a)anthracene (DMBA) powder onto the mammary gland of both 30-day-old female and male Sprague-Dawley rats, and the tumours were examined histologically. Mammary tumours developed in 43/43 (100%) of the females 11 to 20 weeks after DMBA dusting and 16/23 (70%) of the males 18 to 28 weeks after dusting, while non-mammary spindle cell sarcomas occurred in 5/23 (22%) of the males 15 to 24 weeks after dusting. A variety of benign and malignant mammary tumours of epithelial and/or mesenchymal origin were induced, which are comparable to human mammary tumours. Different histological patterns were observed in different areas of the same tumours. Ovariectomy revealed hormone (ovary)-dependency in 10/17 (59%) of the tumours, revealing regressing epithelial and proliferating mesenchymal tumour elements on histological examination.

Key words: Mammary neoplasms – DMBA – Rat – Carcinoma – Sarcoma

Introduction

Mammary tumours are common neoplasms in humans and benign and malignant types demonstrate a variety of histological appearance of epithelial and/or mesenchymal origin. Mammary tumours in rats given polycyclic aromatic hydrocarbons have been extensively studied as a potential model for human breast cancer. In particular, the induction of mammary tumours in female Sprague-Dawley rats by systemic DMBA administration has been studied and single pulse dose administered into either the stomach or caudal vein is the most effective method (Huggins et al. 1961; Huggins 1979). This Huggins tumour is a hormone-dependent adenocarcinoma. Its morphological and biological properties and the endocrine response have been investigated in detail by several workers (Huggins et al. 1961; Young et al. 1963; Archer and Orlando 1968; Murad and von Haam 1972; Tsukidate et al. 1988). The occurrence of sarcomas and mixed epithelial and mesenchymal tumours in the human breast have been described (Azzopardi 1979), but reliable experimental models have not been established. Sinha and Dao (1972, 1974) proposed a technique to induce tumours by the direct application of DMBA powder to the female rat mammary glands. Using this technique, mammary epithelial tumours develop on the dusted site, arising alone, or in co-existence with mammary mesenchymal tumours (Jabara et al. 1979; Morii et al. 1980; Jabara and Anderson 1982; Pacheo-Rupil et al. 1987; Cavalieri et al. 1988). However, the precise histology of the epithelial and mesenchymal elements and the biological behavior of these locally induced tumours have not been reported previously. In this report, the morphological characteristics of mammary tumours induced by the direct application of DMBA powder to the mammary glands of female and male rats are described. Ovariectomy of the tumour-bearing animals was carried out to examine hormone-dependency.

Materials and methods

Sprague-Dawley rats (72 females and 34 males) were purchased from Japan Clea Laboratory, Osaka, after weaning. All of the rats were housed in an air-conditioned room (22±2° C), with

* This work is supported in part by grant-in-aid for cancer research 63010020, from the Ministry of Education, Science, and Culture, Japan

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a daily schedule of 14 h light and 10 h darkness. The rats were housed, 4 to 6 animals per wire-mesh cage, and fed CMF pellets (Oriental Yeast Co., Tokyo, Japan) and water ad libitum.

At 30 days of age, 62 female and 24 males were given 1 mg of DMBA powder by a direct dusting technique as follows: The rat was lightly anesthesized with ether and a small incision was made beside the fifth right inguinal mammary gland. The gland was exposed and dusted with 1 mg of finely powdered DMBA (Eastman Chemical, Rochester, USA). The skin was then flipped back and sealed, using a needle and silk suture. For controls, 10 female and 10 male rats were treated by the same procedure, without DMBA dusting. All rats were palpated once a week to detect mammary tumours. When a tumour appeared, two diameters were measured with calipers, one on the longest axis and the other at a right angle to it. The tumour volume was calculated using the following formula: $(4/3)\pi ab^2$ (a = longest axis, b = right angle to the long axis). The rats were not sacrificed until the tumour had a mean diameter between 1 and 2 cm. No rats died from DMBA dusting, but 2 females and 1 male died of respiratory disease and were excluded from the experiment, which was terminated when the rats were at 32 weeks of age (28 weeks after dusting). Complete autopsies were performed on all of the rats.

To carry out histological examination, the tumour, together with the adjacent normal mammary gland, was fixed in 10% neutral buffered formalin and embedded in paraffin. The contralateral inguinal mammary gland of the dusted rats and mammary glands of rats which had not undergone DMBA dusting was processed in the same manner, serving as controls. Tissue sections were stained with haematoxylin-eosin (H-E), alcian blue (pH 2.5) and perodic acid-Shiff (PAS).

Small pieces of tumour tissue were fixed in 3% glutaraldehyde in 0.01 M phosphate buffer, pH 7.4, for 1–2 h at 4° C and overnight wash in phosphate buffer, followed by post-fixation with 2% osmium tetroxide. Blocks of tissues were then dehydrated in a graded series of ethanol, embedded in Luveak-812. Ultrathin sections were cut and double stained with uranyl acetate and lead citrate in a routine manner. Sections were examined under a Hitachi-500 electron microscope.

Bilateral ovariectomy was performed on 17 rats with finger tip-sized tumours. Change in volume was expressed as a percentage of the original volume; the size of each tumour was measured weekly. Those tumours showing a reduction in size of greater than 20% were designated hormone-dependent, and those showing no reduction or an increase in size, designated hormone-independent. These rats were sacrificed 1 to 3 weeks after ovariectomy.

Results

The 1–2 cms diameter tumours occurred on the dusted site in 43 female rats (100%; 43/43), and in 21 male rats (91%; 21/23). This occurred 11 to 20 weeks after dusting in females, with a mean latency of 15.6 weeks, and 15 to 28 weeks after dusting in males, with a mean latency of 22.0 weeks.

The tumours were classified to the following 2 groups: mammary, where the mammary parenchymal cells were either proliferating atypically or were engulfed by the atypically proliferating mesenchymal cells, and non-mammary in origin, where the tumour tissue was not connected to periductal/intralobular fibrous stroma. All of the tumours in the females were mammary in origin, while in the males, 16 tumours were mammary in origin and 5 tumours were non-mammary. The time of harvest and cumulative incidences of mammary and non-mammary tumours in both sexes are shown in Fig. 1. No metastasis from the tumours was detected histologically in any of the rats examined. Contralateral mammary tissues revealed no neoplastic change. Precancerous changes, such as hyperplastic alveolar nodules, were not seen in any mammary tissue examined. Other tumours, e.g. leukaemia or ear duct tumour, were not evoked. All undusted control rats, autopsied at 32 weeks of age, were free or tumours.

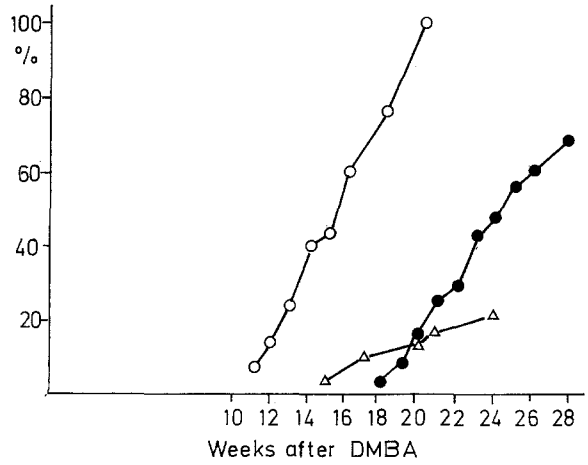

Fig. 1. Cumulative incidences and latency of tumours after direct dusting with DMBA powder to the mammary gland of 30-day-old Sprague-Dawley rats. (o——o; female rats with mammary tumour, ●——● male rats with mammary tumours, △——△; male rats with non-mammary tumours)

The mammary tumours were heteromorphic with a mixture of various histological appearances (Table 1). In general, 1 to 4 histological patterns were seen in different areas of the same tumour. In the papillary adenocarcinomas, a prominent epithelial outgrowth showed many papillary projections composed of one to several layers due to the piling up of the inner lining duct cells. The adenoid cystic carcinomas revealed a sheet of tumour cells separated by small cystic spaces, which were filled with either PAS-positive secretory materials or alcian blue-positive ones. In addition to these carcinomas commonly seen in Huggins tumour, a squamous cell carcinoma (Fig. 2) was often located in the center of the tumour where necrotic changes and residual DMBA crystals were observed. Adenosquamous carcinomas (Fig. 3) were also seen around the metaplastic areas of ducts which had come into contact with DMBA

Table 1. Frequencies of histological pictures of induced tumours by direct application of DMBA powder in 30-day-old Sprague-Dawley rats

Number of animals	43 females	23 males	
Tumour incidence (%)	100	91	
Number of finger tip-sized tumours	mammary 43	mammary 16	non-mammary 5
Epithelial tumours			
papillary adenocarcinoma	29*	3	–
adenomatosis	16	1	–
squamous cell carcinoma	14	4	–
intraductal papilloma	10	0	–
adenoid cystic carcinoma	8	0	–
adenosquamous carcinoma	3	0	–
Mesenchymal tumours			
fibromatosis	21	9	0
fibrosarcoma	14	11	5
Mixed epithelial and mesenchymal tumours			
fibroadenoma	10	1	–
carcinosarcoma	4	6	–
phyllodes tumour	3	0	–

* Number of lesions observed

powder. Adenomatosis and intraductal papilloma (Fig. 4) were also seen.

Apart from these epithelial tumours, a high percentage of the mammary mesenchymal tumours, and mixed epithelial and mesenchymal tumours were induced. There were stromal sarcomas (Fig. 5), fibroadenoma, phyllodes tumour (Fig. 6) and carcinosarcoma (Fig. 7). Most of the neoplastic mesenchymal cells were spindle-shaped (Fig. 8a), some were oval-shaped cells with eccentrically located nuclei (Fig. 8b) or large polygonal eosinophilic cells with bizzare nuclei (Fig. 8c).

In males the mammary tumours were, in general, the same as those seen in females. Non-mammary spindle cell sarcomas were also seen, probably originating from the skin or body wall at the side of dusting.

On electron microscopy, fully differentiated myoepithelial cells were not detected. Cytoplasmic filaments were reduced. These filaments were short and ran in various directions. It is interesting that 2 types of lumina were observed in the adenoid cystic carcinoma. One was characterized by the presence of microvilli (Fig. 9), the other was covered with a layer of myoepithelial cells accompanied by basal lamina with irregular thickenings (Fig. 10). Neoplastic stromal cells demonstrated either fibroblastic or myofibroblastic differentiation. The cytoplasm of cells with fibroblastic differentiation contained many polysomes, mitochondria, dilated rough endoplasmic reticulum and a Golgi apparatus. Those cells of myofibroblastic differentiation were defined by a linear array of 70 Å filaments (Fig. 11). Electron-dense membrane-bound bodies thought to be lysosomes tend to increase in atypical-shaped cells. Z-lines, liposomes and Weibel-Palade bodies were not seen.

Seventeen female rats carrying 1–2 cm diameter mammary tumours were ovariectomized. The tumours were classified into 10 hormone-dependent, and 7 hormone-independent cases. The former were defined by a definite reduction in size and softening. Morphology revealed flattening of the degenerating glandular and myoepithelial cells and enlargement of the lumina. Such atrophic changes were not usually demonstrated in the entire tumour tissue and some epithelial components were still actively growing. Regressive changes never occurred in the sarcomatous elements (Fig. 12).

Discussion

The present experiment clearly demonstrated that a direct dusting of DMBA powder in 30-day-old rats could evoke a locally induced mammary tumour with a high yield. The latency period was prolonged compared to the Huggins tumour in-

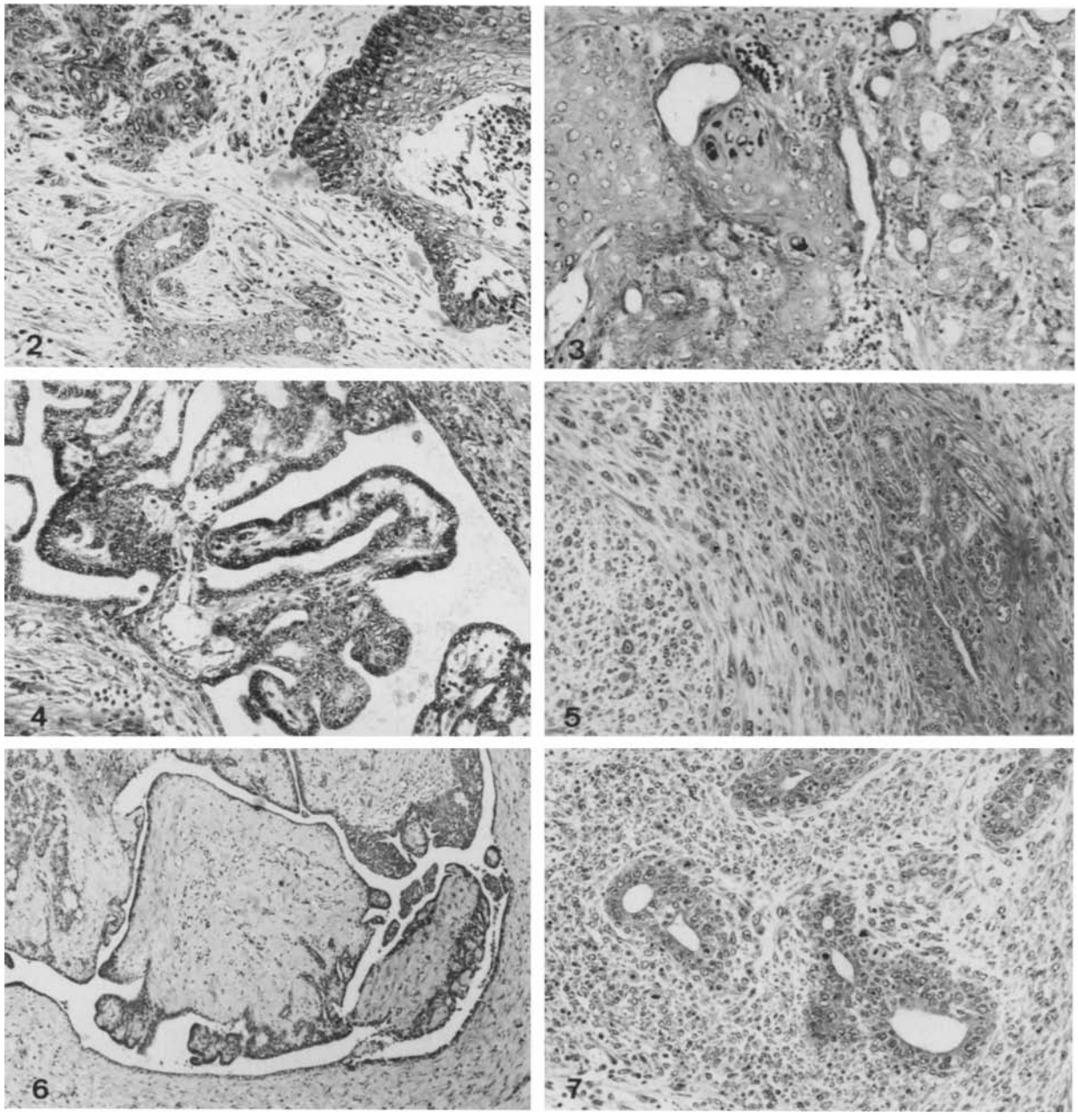

Fig. 2. Squamous cell carcinoma; inflammatory cells are frequently seen. Cancer cells are often found near the transformed mammary ductal cells. H-E × 200

Fig. 3. Adenosquamous carcinoma; cell with keratohyalin granules are seen in connection with adenocarcinoma cells. H-E × 200

Fig. 4. Intraductal papilloma; benign papillary projections accompany stroma. H-E × 200

Fig. 5. Stromal sarcoma; proliferation of atypical stromal cells. Note normal mammary ducts are engulfed at the periphery. H-E × 200

Fig. 6. Phyllodes tumour; slit-like spaces are lined by epithelial cells. Stromal cellularity is moderate. H-E × 100

Fig. 7. Carcinosarcoma; atypical cells with mitosis seen in both epithelial and stromal component. H-E × 200

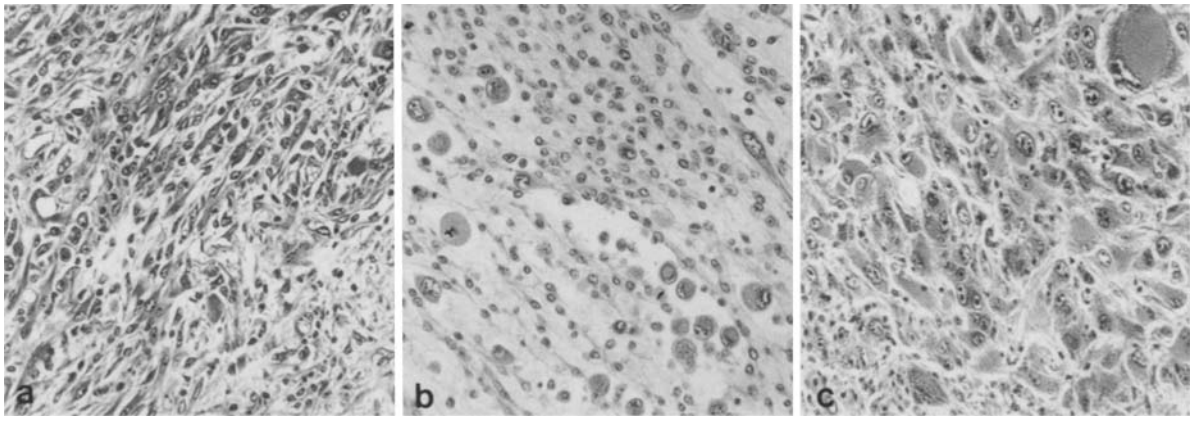

Fig. 8. Mesenchymal cells; majority of mesenchymal cells are spindle-shaped (**a**), while some are oval-shaped (**b**) or show large polygonal cytoplasm (**c**). H-E each ×240

duced by systemic administration of DMBA (Huggins 1979). There are several differences in the biology of the tumours induced using the two different techniques.

Firstly, preneoplastic changes were numerous in the mammary glands of all rats receiving DMBA administration systemically, but tumours only developed in the mammary gland where DMBA had been applied in the direct model (Sinha and Dao 1972, 1974, 1975; Jabara et al. 1979; Morii et al. 1980; Jabara and Anderson 1982; Pacheco-Rupil et al. 1987; Cavalieri et al. 1988). Sinha and Dao (1974) reported that microscopic tumours originated in the duct as early as 30 days after the local application of DMBA without the previous existence of hyperplastic alveolar nodules. When the target cells are exposed directly to DMBA for a longer time, then they are probably transformed into neoplastic cells directly, without any intermediate steps (Sinha and Dao 1974, 1975). Benign tumours tend to arise later than malignant ones following systemic administration (Daniel and Pritchard 1964). No correlation was seen in the present study. Therefore, local carcinogenesis might be less affected by general factors in the hosts, for example, age, sex and hormonal status.

Secondly, the dusting technique eliminated any observable systemic effects of DMBA, such as adrenal apoplexy (Huggins and Morii 1961) which markedly reduced the mortality of the experimental animals.

Thirdly, the direct dusting affected not only the mammary epithelial cells but also the mesenchymal cells at the dusting site, while systemic administration of DMBA rarely evoked sarcomatous changes (Huggins 1979). Almost all Huggins tumours are adenocarcinomas, but adenosquamous carcinoma and squamous cell carcinoma were seen in the present experiment. Murad and van Haam (1972) described some well differentiated myoepithelial cells in DMBA induced mammary carcinomas, but in agreement with Ormerod et al. (1985) and Tsukidate et al. (1988) were unable to see fully differentiated myoepithelial cells. In adenoid cystic carcinoma, in addition to peripherally-located cells, some luminal surrounding cells with a few of the properties of myoepithelium could be seen. In DMBA induced mammary tumours, radioautography using tritiated thymidine showed proliferating cells in the stroma as well as epithelial cells (Murad and von Haam 1972). The importance of the stroma in carcinogenesis has been discussed by Ozzello (1970), and it is quite possible that the stromal connective tissues play an important role in the survival and growth of epithelial tumours. With the direct dusting technique, some mammary mesenchymal cells transformed directly into neoplastic cells, and they were mainly fibroblastic in nature. Sometimes, neoplastic proliferations of myofibroblasts could be identified on electron microscopy. These changes are comparable to those in fibromatosis (Wargotz et al. 1987a), myofibroblastoma (Wargotz et al. 1987b) or fibroadenoma (Ohtani and Sasano 1984) of the human breast. Fibrosarcoma cells revealed an increase of dense membrane-bound bodies thought to be lysosomes and an increased number of these bodies may reflect increased cell turnover (Harris and Khan 1984; Yeh et al. 1985). The cells were confirmed to be of mesenchymal origin by their vimentin positivity (Tsubura and Morii 1988). Heterologous differentiation forming bone, cartilage or fat cell, which were described in human breast (Norris and Taylor 1968), was not obvious.

Fourthly, in males, although the incidence of tumours was low and the latency was longer than

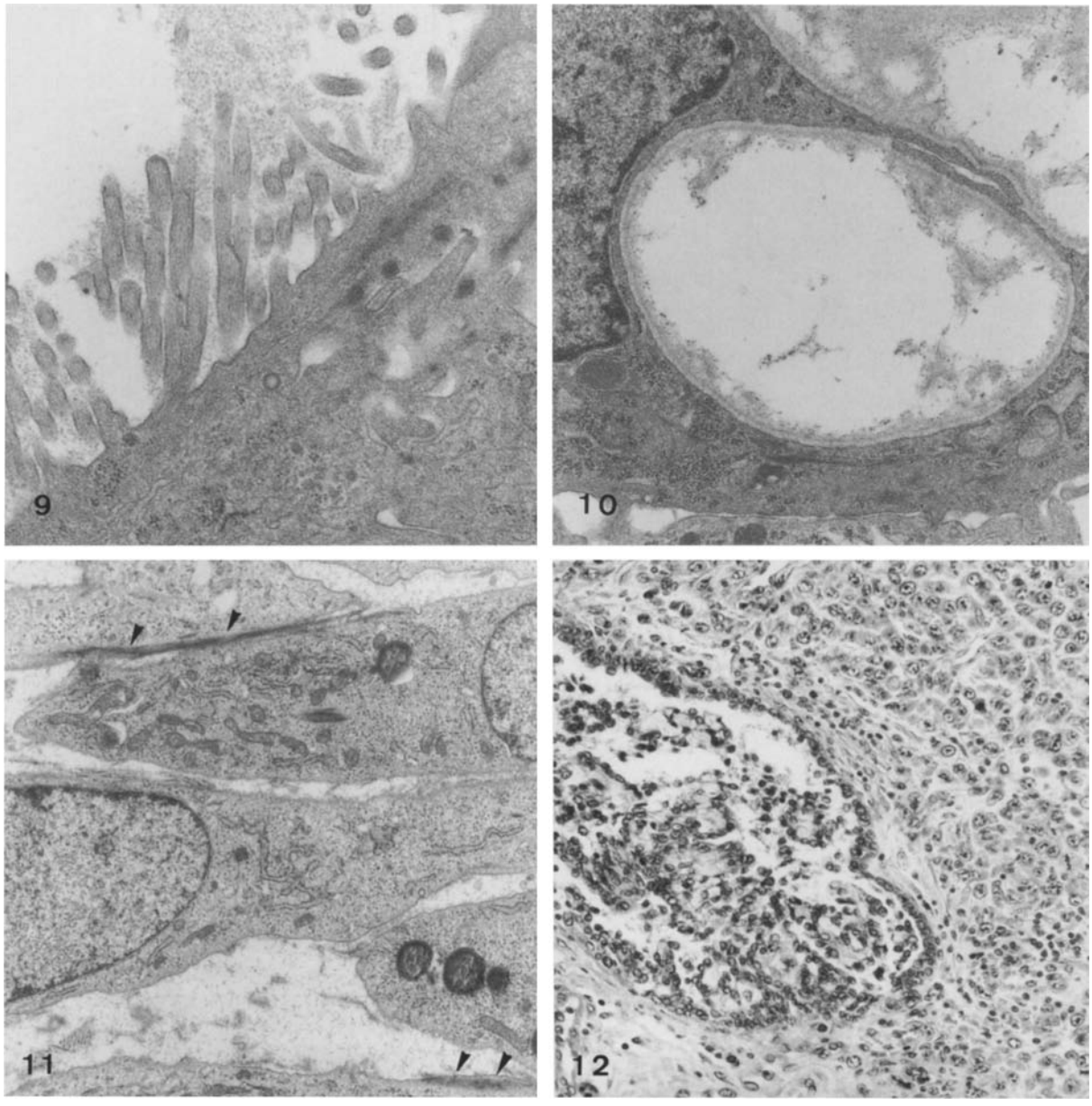

Fig. 9. Adenoid cystic carcinoma; microvilli are seen in some lumen. × 24000

Fig. 10. Adenoid cystic carcinoma; the other lumen is surrounded by irregular basal lamina and myoepithelial cells. × 13500

Fig. 11. Stromal component; majority of stromal cells are spindle shaped. Myofibroblast is seen containing 70 Å filaments (*arrow head*). × 5000

Fig. 12. Carcinosarcoma; flattening of the epithelial cells and condensation of their nuclei indicates degeneration of the epithelial elements, while no degenerative changes are seen in sarcomatous components (compare with Fig. 7). Macroscopically, this tumour was designated hormone-independent. H-E × 360

in females, mammary tumours may have been evoked by this direct dusting technique. Non-mammary tumours, composed of mesenchymal cells, were induced only in males. Male rats are more refractory than females to the induction of mammary tumours by systemic administration of DMBA (Huggins and Grand 1966) and tumours were induced in males in high yields only when 8 repeated pulse doses were given (Yoshida et al. 1982).

Fifthly, some of the locally induced mammary tumours in females showed regressive changes, after bilateral ovariectomy, similar to Huggins tumour (Young et al. 1963). Histologically, the tumour cells which responded to ovariectomy were the epithelial component and the mesenchymal cells were not affected.

In conclusion, the local application technique evokes mammary tumours at the dusting site in both sexes, at high incidence. Although the classification of rat mammary tumours is arbitrary (Young and Hallowers 1973; Komitowski 1982), the tumours partly resembled human breast neoplasms and some of the epithelial elements regressed after ovariectomy. The results of our study may offer a new model for understanding human breast neoplasms.

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Received June 27, 1988 / Accepted August 18, 1988