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Tumors Developing in Oophorectomized Sprague-Dawley Rats after a Single Gastric Instillation of 7,12-Dimethylbenz(*a*)anthracene[1]

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SUMMARY

The present report describes the various types of tumors which developed in female Sprague-Dawley rats oophorectomized at age 46 days and fed a single dose of 20 mg 7,12-dimethylbenz(*a*)anthracene one week later. This method, which is known to induce breast carcinoma invariably in normal female rats of this strain, produced mammary cancer in only 5% of the oophorectomized rats, after a period of observation of about a year.

In contrast, a high percentage of rats presented tumors of the ear duct (59%), neurofibrosarcomas of the ear lobe (64%), and various tumors of the skin and skin appendages (22%). The appearance of these extramammary tumors may be due in part to the longer lifespan of the rats because they escaped the lethal effects of breast carcinoma. However, a relationship between carcinogenic reactivity of the skin and the endocrine status of the host cannot be ruled out.

INTRODUCTION

The remote administration of various carcinogens induces hormone-dependent mammary carcinomas in the female rat. These tumors regress after oophorectomy, hypophysectomy, or testosterone administration; they rarely metastasize, but become very large, infiltrate the adjacent soft tissues, ulcerate, and eventually kill their hosts (6). Huggins *et al.* (9) have shown that a single gastric instillation of DMBA[2] or MCA to female Sprague-Dawley rats induces multiple mammary tumors invariably when these carcinogens are given under definite conditions of age and dosage.

Various extramammary tumors have been reported incidentally in works dealing with carcinogen-induced mammary cancers (9, 10, 11, 12). In the present work, we have studied a series of rats which were oophorectomized prior to a single instillation of DMBA. The majority of these animals did not develop mammary cancer and therefore escaped the lethal effect of this malignancy. However, other tumors emerged; it is the purpose of this report to describe them and to discuss their possible relationship with the endocrine status of their hosts.

MATERIALS AND METHODS

A total of 64 Sprague-Dawley rats was studied. These animals were oophorectomized at age 46 days and given a single gastric instillation of 20 mg of DMBA, dissolved in 1 ml of sesame oil, 1 week later. Between 7 and 9 months after carcinogen administration, 11 animals died or were killed because they looked ill. All the other rats were sacrificed 12 months after DMBA instillation. An autopsy was performed on all 64 rats and representative fragments from all tumors were excised; they were fixed in 15% formalin and embedded in paraffin. The sections were stained with hematoxylin and eosin for histologic examination.

RESULTS

Breast Tumors. Thirteen mammary tumors were found in 12 rats (Table 1). Three rats (5%), all belonging to the group that died before the end of the experiment, had one mammary carcinoma each. These tumors were highly cellularized and poorly differentiated adenocarcinomas. Ten fibroadenomas were found in 9 rats (14%). These tumors frequently reached several centimeters in diameter and displayed the classical pattern of benign glandular proliferation, surrounded by dense fibroblastic or hyalinized stroma.

Tumors of the Ear Duct (Tumors of Zymbal's Gland). Thirty-eight rats (59%) developed unilateral (36%) or bilateral (23%) tumors of the ear duct (Table 1). These tumors were first noticed 5 months after administration of DMBA when they had reached an externally visible size. One-third of these lesions were occult tumors discovered only at autopsy when the neck was dissected. The tumor was found in 5 of the 11 rats which died before the end of the experiment.

The gross aspect varied from a small nodule measuring about 0.5 cm to a large lobulated and cystic mass filled with yellowish-green frosty material. The larger tumors measuring several centimeters, frequently adhered to the adjacent soft and bony structures of the neck, and ulcerated through the skin. On microscopic examination, the cystic pattern corresponded to multiple and large cavities partially filled with keratin or sebum (Fig. 1). The lining epithelium showed papillomatous

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[2] The following abbreviations are used: DMBA, 7-12-dimethylbenz(*a*)anthracene; MCA, 3-methylcholanthrene; 2-AAF, 2-acetylaminofluorene; 2-AF, 2-aminofluorene; TACP, tris(p-aminophenyl)carbonium pamoate.

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projections into the lumen and cords of cells budding outwards in the surrounding stroma. In the majority of cases, the lesions were interpreted as adenomas because the lining epithelium was made of well-differentiated squamous or sebaceous cells with only moderate inflammatory changes.

Around one-fifth of the tumors were definite carcinomas: the cellular arrangement around the cavities was anaplastic (Fig. 2); instead of the round and clearly demarcated buds seen in adenomas, there were irregular strands of malignant cells with hyperchromatic nuclei and atypical mitoses invading the stroma. An important inflammatory infiltrate and a strong desmoid reaction were present. A few tumors exhibited a pseudosarcomatous pattern. One of them had metastasized to the cervical lymph nodes and to the lung. All the carcinomas were large and ulcerated, but some of the largest tumors were histologically benign.

Epidermal Tumors. Under this heading, 17 cutaneous lesions found in 14 rats (22%) are described. They usually appeared as small, hard, single cutaneous nodules less than 1 cm in diameter, but some were large ulcerated tumors. Twelve single tumors were located along the breast areas. Microscopic examination showed a broad spectrum of various tumors involving the skin and its appendages. They could be divided into 6 epidermal cysts, 4 keratoacanthomas, 2 sebaceous epitheliomas, 2 basal cell epitheliomas, 1 wharty papilloma, 1 sebaceous cyst, and 1 trichoepithelioma.

In addition, 2 rats showed abdominal nodules which turned out to be fibrous scars originating, probably, from self-healed epidermal tumors or from regressed mammary tumors. Further evidence that several lesions had developed and regressed spontaneously during lifetime was brought out by the fact that more nodules were actually observed during the periodic examinations than were ultimately counted at the end of the experiment: the same 12 rats which presented single lesions along the breast lines at autopsy had 32 nodules in the same regions when checked at 7 months after DMBA instillation.

These tumors were first observed during the 5th month following carcinogen administration.

Neurofibrosarcomas. Five months after DMBA administration small, round, soft nodules appeared on one or both ear lobes of several rats. These tumors grew slowly and reached a diameter of 0.8 to 1 cm (Fig. 3). Frequently 1 or 2 additional nodules developed later and coalesced with the first one to form a multinodular, superficially ulcerated, pink tumor with a glittering cut surface. Forty-one rats (64%) eventually showed this lesion (Table 1). In 11 rats (17%), it was bilateral. In addition, one of these rats presented a similar lesion on the nose and another on the tail.

Histologic examination revealed a well-circumscribed upperdermal tumor formed by interlacing highly cellularized bundles of spindle-shaped cells (Fig. 3). In some areas, a palisading pattern was observed. The nuclei were irregular and hyperchromatic and mitoses were occasionally present (Fig. 4), but metastases were not encountered.

Other neoplastic lesions. Two cases of leukemia, 2 cases of low-grade dermal fibrosarcoma of the neck, and one retroperitoneal fibrosarcoma were also detected.

DISCUSSION

It has been known for many years that oophorectomized rats are much less prone than intact females to develop mammary cancer after carcinogen administration (2, 14). Our results are in accordance with this fact. Whereas a single feeding of 20 mg DMBA to intact female rats induces multiple mammary tumors in 100% of the animals (9) only 5% of our oophorectomized rats developed mammary carcinoma. Huggins *et al.* (9), using a single massive dose of MCA which was effective in 100% of the intact controls, also failed to induce any mammary carcinoma in castrated females. In contrast, by feeding MCA 6 times a week for several months, they produced the lesion in 74 of 108 rats which had been oophorectomized at age 42 days (8). It would therefore appear that, while a single feeding of a carcinogen to intact female rats is nearly as effective as multiple administrations, in castrated females, repeated administration is needed in order to produce mammary carcinoma.

Tumors of the ear duct were found at a surprisingly high frequency (Table 1), considering that only a single dose of carcinogen was given. In the literature, the lesion has been reported to occur several months after administration of various carcinogens: 2–AAF and 2–AF (1, 15), MCA (9, 11), DMBA (6, 10), urethan (16), anthramine (17) and, more recently, TACP (13) and Ibenzmethyzin (Natulan^{R}) (4). However, a high incidence of this tumor was reported only when the carcinogen was given for long periods of time: 66% in a series of Kim and Furth (11), who gave repeated instillations of MCA during 6 to 14.5 weeks; 57% in the experiments of Skoryna *et al.* (15), who added 2–AAF to the diet for 15 weeks; and 49, 5% by Schardein and Kaump (13), who incorporated TACP into the diet. When DMBA was given in a single dose of 20 mg, as was done here, the incidence reported by Huggins was as low as 5% (10).

The higher incidence found in our series is most probably related to the longer lifespan of the oophorectomized rats. The particular attention paid to this lesion and the systematic dissection of the neck carried out in order to disclose occult tumors may have been a contributory factor. However, one wonders whether oophorectomy by itself might not have influenced the frequency of these tumors. The data in the literature concerning the sex distribution of the ear duct tumor are rather conflicting. Skoryna *et al.* (15), who studied an equal number of males and females, found a sex distribution of 43 females to 29 males, but they did not take this sex distribution into account because the males had a higher mortality in the early months of carcinogen administration. Tannenbaum *et al.* (16) reported similar incidences in both sexes. Schardein and Kaump (13) claimed a sex difference, finding 5 times more ear duct tumors in female than in male rats. Gruenstein *et al.* (3), on the other hand, reported a clear male preponderance of tumors in or near the area of Zymbal's gland. It must be stressed, however, that the lifespan of the female rats was much shorter in this latter study.

It remains to be seen how much in these discordant reports is actually due to the use by these authors of different strains and different carcinogens. Be that as it may, our data indicate that, under the present conditions, intact ovarian function was

Table 1

Type of tumor	No. of tumors	No. of rats	% of rats	Rats with bilateral tumors	
				No.	%
Total mammary tumors	13	12	19		
Carcinomas	3	3	5		
Fibroadenomas	10	9	14		
Total tumors of the ear duct	53	38	59	15	23
Benign	46	31	48		
Malignant	7	7	11		
Total epidermal tumors	17	14	22		
Epidermal tumors in the breast areas	12	12	19		
Total neurofibrosarcomas	54[a]	41	64		
Neurofibrosarcomas on the ear lobes	52	41	64	11	17
Other sarcomas	3	3	5		
Leukemia		2	3		

Type and incidence of tumors found at autopsy in 64 Sprague-Dawley female rats oophorectomized at age 46 days and fed a single dose of 20 mg DMBA 1 week later.

[a] Two rats carrying neurofibrosarcomas on the ear lobes presented similar lesions elsewhere.

not required for induction and growth of these tumors; moreover, a single feeding of carcinogen was as effective in inducing these lesions as any other known means involving the repeated administration of carcinogen over long periods of time.

It seems that a distinction has to be made between the ear duct tumor and the other epidermal tumors; for the latter, the literature is more concordant with references to sex distribution. Tannenbaum *et al.* (16) reported the appearance of epidermal cysts more generally on the back and the flanks in male rats after urethan administration, whereas none was observed in the control males or the treated and control females. In the work of Gruenstein *et al.* (3), the incidence of cutaneous lesions is also much lower in female than in male rats, and these authors raise the possibility that in the rat the neoplastic reactivity of the skin and its appendages could be almost as sex specific for males as is the breast for females. The fact that 22% of our oophorectomized rats presented similar lesions would be consistent with this assumption. We have not found any report about these lesions in works dealing with administration of a single dose of DMBA to female rats. It is known, however, that when carcinogens are used as topicals, there is no sex difference in the distribution of epidermal tumors (5, 17), but, as Gruenstein *et al.* (3) pointed out, this may be related to the route of administration.

A remarkably high incidence of neurofibrosarcomas of the ear lobe was observed in this material (Table 1). Tannenbaum *et al.* (16) discovered this lesion after repeated administration of urethan in 4% of their rats. In a recent work, Huggins and Grand (7) reported 2 ear sarcomas found at the site of perforation made for identification; they ascribed them to the trauma which had localized the carcinogen (DMBA injected intravenously). Such a mechanism cannot be invoked in our series.

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Fig. 1. Adenoma of Zymbal's gland. The picture shows a cystic cavity lined by a benign epithelium. A clear demarcation can be seen between the epithelial cells and the stroma. H & E, × 160.

Fig. 2. Carcinoma of Zymbal's gland. A cystic pattern is recognizable. The epithelium around the cavity is anaplastic and fades into the stroma which shows a strong desmoid reaction. H & E, × 240.

Fig. 3. Neurofibrosarcoma. The gross aspect of the tumor is seen in the *inset*. H & E, × 100.

Fig. 4. Neurofibrosarcoma. The tumor cells are spindle-shaped; the nuclei are irregular and hyperchromatic; mitoses are easily seen. H & E, × 700.